1985

Strawberry Response to Collectotrichum Fragariae and Colletotrichum Acutatum (Anthracnose, Crown Rot).

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STRAWBERRY RESPONSE TO *COLLETOTRICHUM FRAGARIAE* AND *COLLETOTRICHUM ACUTATUM*

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Pathology and Crop Physiology

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August 1985

ACKNOWLEDGEMENTS

The author wishes to express appreciation to Dr. Lowell L. Black, Professor of Plant Pathology, for his guidance and assistance in conducting this research and for his counsel and suggestions in the preparation of this manuscript.

Thanks are expressed to the members of my committee for their advise and constructive criticisms regarding this manuscript.

Gratitude is expressed to Dr. Carleton D. Ranney, Area Director, Mid-South Area of the Agricultural Research Service of the United States Department of Agriculture and to Dr. James M. Spiers, Research Leader of the USDA-ARS Small Fruit Research Station in Poplarville, MS for the funding which made my training and this research possible.

A very special thanks goes to my parents, Mr. and Mrs. W. W. Jones, and to my husband's parents, Mr. and Mrs. Albert Smith, Jr., for their encourgement and support during this endeavor.

Finally my deepest gratitude goes to my husband, Robert, and our two sons, Robert D. and Scott, for the motivation, constant encouragement and understanding they furnished as well as the sacrifices they made.

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ABSTRACT

Colletotrichum fragariae Brooks, the causal agent of strawberry anthracnose (referred to herein as anthracnose-crown rot), has been reported to infect all above ground parts of the strawberry plant. Colletotrichum acutatum Simmonds is known to cause ripe rot of several fruit including strawberry. Twenty-four Colletotrichum isolates obtained from strawberry plants from several southeastern states and California were compared. Based on conidial morphology and cultural characteristics, 15 of the isolates were identified as C. fragariae and nine as C. acutatum. Two of the C. acutatum isolates were obtained from field grown plants exhibiting crown rot symptoms. This is the first report of C. acutatum causing a crown rot of strawberry plants.

A wide range in pathogenic variation was evident among isolates of both C. fragariae and C. acutatum. In general, C. fragariae isolates caused more severe petiole and crown symptoms than C. acutatum isolates. However, some cultivars were more susceptible to certain C. acutatum isolates than to some C. fragariae isolates. Most of the C. fragariae and some of the C. acutatum isolates caused a crown rot on certain cultivars.

Inoculation methods and conditions were compared. A plant spray inoculation method was best for evaluation of petiole response to C. fragariae. However, crown injection was necessary to reliably assess the crown rot response. Incubation in a dew chamber at 32 to 35 C for 48 hr immediately following inoculation was necessary for rapid disease development. Increasing the temperature from 25 to 32 C in the

greenhouse in which inoculated plants were held resulted in substantially more severe disease symptoms on some cultivars tested.

Two- to 4-wk-old seedlings (age after transplanting at the first-true-leaf stage) were in general more susceptible to C. fragariae than 14- to 18-wk-old seedlings; however, three seedling populations expressed juvenile resistance. More than half the strawberry seedlings derived from crosses involving previously reported anthracnose-crown rot resistant parents were found to be resistant to the crown rot phase in crown injection tests. Plants of most commercial cultivars were very susceptible to crown injection by C. fragariae and died within 50 days of inoculation.

Chapter I

INOCULATION METHODS FOR IDENTIFICATION OF RESISTANCE IN STRAWBERRY TO *COLLETOTRICHUM FRAGARIAE*

INOCULATION METHODS FOR IDENTIFICATION OF RESISTANCE IN STRAWBERRY TO *COLLETOTRICHUM FRAGARIAE*

Colletotrichum fragariae Brooks, the causal agent of strawberry anthracnose (referred to herein as anthracnose-crown rot), may attack any of the above ground parts of the strawberry (*Fragaria* X *ananassa* Duch.) (1, 2, 7, 8, 9), but the fungus is most devastating when it invades the crown which results in a wilt and sudden death of the plant (7, 10). To have a name that distinguishes the disease caused by *C. fragariae* from those caused by other *Colletotrichum* spp., the name 'anthracnose-crown rot' is proposed. The crown rot phase of the disease is most severe during warm, humid conditions and frequently causes damage in both fruit production fields and summer nurseries in the Gulf Coast states and in summer nurseries in other southeastern states (10). Resistance to *C. fragariae* in a few cultivars including Apollo, Dover, Florida Belle, Rosanne and Sequoia has been reported (3, 11). However, some of these cultivars have been shown to be susceptible to certain isolates of *C. fragariae* in greenhouse tests (4, Chapter 2). In a project to develop anthracnose-crown rot resistant cultivars adapted to the southeastern United States, the United States Department of Agriculture, Agricultural Research Service, has been conducting a strawberry breeding program at Beltsville, MD and Poplarville, MS in cooperation with state agricultural experiment stations (14).

To make rapid progress in identifying resistant germplasm and in incorporating known resistance into commerical cultivars, a reliable greenhouse disease resistance screening technique is needed. Previous reports on greenhouse methods for evaluation of strawberry plant resistance to C. fragariae have left questions concerning the inoculation method and the incubation conditions that should be used following inoculation. Delp and Milholland (3) reported that inoculum entering the crown of the plant during greenhouse inoculation allowed the pathogen to bypass resistance in the strawberry lines with which they were working. They recommended a distal petiole inoculation method in which petiole lesion size is used to evaluate the petiole response of strawberry clonal lines to C. fragariae. In a separate study, Smith and Spiers (14) demonstrated resistance to C. fragariae in strawberry seedlings using a spray inoculation technique in which no attempt was made to keep inoculum out of the crown. Their results suggested that resistance to the crown rot phase of the disease existed in some of their strawberry lines. Because crown rot is the most destructive phase of the disease in the Gulf Coast states, the present study was initiated to compare various inoculation methods for assaying strawberry seedlings, cultivars, and breeding lines for resistance to C. fragariae with particular emphasis on identification of strawberry sources resistant to the crown rot phase.

The importance of high temperature (>25 C) and humidity (RH near 100%) for disease development of strawberry plants infected with C. fragariae has been well documented both in the field (1, 7) and in the greenhouse (1, 3, 7, 14). Incubation in an atmosphere of near 100% RH for at least 48 hr immediately after inoculation is necessary for good

disease development (1, 3, 12, 14). However, no reports could be found on the effect of temperature on disease development during this initial incubation period. Delp and Milholland (3) found that plants held at higher greenhouse temperatures after removal from the moisture chamber developed more severe disease symptoms than plants held at lower temperatures. They considered a greenhouse temperature of 25 C optimum for evaluation of petiole lesion symptoms on cultivars and a greenhouse temperature of 30 C capable of breaking resistance. They also determined that an inoculum density of 10^6 conidia per ml was optimum for disease assessment and that an inoculum density of 10^7 could overcome resistance. A part of the present study was designed to refine the inoculation conditions necessary to reliably assess resistance, including resistance to the crown rot phase of anthracnose-crown rot, in strawberry cultivars and seedlings to infection by C. fragariae. The effect of inoculum concentration, dew chamber temperature, and greenhouse temperature on infection of three cultivars by two isolates of C. fragariae were evaluated.

The effect of plant age on seedling response to inoculation with C. fragariae has not been established because in most previous studies strawberry runner plants of clonal lines have been evaluated for their reaction to C. fragariae (1, 3, 4, 7). Horn et al. (6) used seedlings in a study of anthracnose-crown rot resistance, but they did not report the age of the seedlings used in the study. Smith and Spiers (14) inoculated seedlings 6 to 8 wk after transplanting at the first-true-leaf stage but did not compare this age seedling with other age seedlings. A part of the present study was designed to determine if adult resistance of strawberry plants to C. fragariae is expressed in the juvenile stage and

if not, what age seedlings must be utilized in a screening program to assess adult plant resistance.

MATERIALS AND METHODS

Strawberry seedlings. "Seedling" is used in this paper to refer to unselected progeny of strawberry populations grown from seed. "Breeding line" refers to a numbered clonal line selected and maintained vegetatively for use in a breeding program. "Cultivar" is a named clonal line available to the public. Strawberry seed derived from 13 crosses made among 21 clonal lines by G. J. Galletta at Beltsville, MD (Table 1) were germinated on ground spaghnum in a growth chamber at 24 C (day) and 18 C (night) with a 16 hr photoperiod (Sylvania, T12-GRO-VHO Daylight fluorescent and 40 W incandescent lights) (13). Seedlings were transplanted to Jiffy-7 Peat Pellets (Jiffy Products, Ltd., Norway) at the first-true-leaf stage. Strawberry seeds do not germinate uniformly, therefore, to have plants at a uniform stage of development for inoculation studies, seedling age was designated in this study as the time after transplanting seedlings at the first-true-leaf stage. The seedlings were grown in a greenhouse with supplemental lights (General Electric F400 Daylight fluorescent and 40 W incandescent) to achieve a 16 hr photoperiod with day temperatures of about 22 C and night temperatures of about 18 C. Weekly applications of Peters (Robert B. Peters, Inc., Allentown, PA) soluble fertilizer (10-30-20) at the rate of 0.3 g/liter were made beginning 3 wk after transplanting. Older leaves and runners were cut off all seedlings one to 7 days before inoculation leaving two to four young leaves on the plant. Wounding at the time of inoculation

Table 1. Strawberry seedling populations and the reported anthracnose-crown rot response of their parent lines

Code	Parent Lines	Anthracnose Designation[a]
82-78	MSUS 27 X LA 7113A	R_2[b,c] X R_2[b,d]
82-79	MSUS 31 X FLA 73-1872	R_2[b,c] X R[e]
82-80	LA 7525A X US 78-1760AN	R_2[b,d] X R_2[b,e]
82-84	FLA 76-577 X NC 3920	R[e] X R[f]
82-85	US 78-1839AN X LA 7517A	R_2[b,e] X R_2[b,d]
83-65	LA 883 X Dover	R[d] X VR[g]
83-66	MSUS 27 X US 78-1760AN	R_2[b,c] X R_2[b,e]
83-67	Prelude X Dover	S[g] X VR[g]
83-68	Atlas X Florida Belle	S[g] X R[g]
83-70	LA 883 X Olympus	R[d] X U
83-72	MSUS 42 X MDUS 5146	R_2[b,c] X U
83-73	LA 7922A X Douglas	R_2[b,d] X VS[g]
83-76	Florida Belle X Pajaro	R[g] X VS[g]
Tioga O.P.	Tioga Open Pollinated	VS[g] X VS[g]
Tufts O.P.	Tufts Open Pollinated	VS[g] X VS[g]

[a]Reported anthracnose-crown rot response of parent lines: VS = very susceptible; S = susceptible; R = resistant in field; R_2 = resistant in greenhouse screening and in field; VR = very resistant in field; U = unknown.

[b]Selected as resistant in greenhouse screening at Poplarville, MS.

[c]Selected as resistant in field at Poplarville, MS.

[d]Selected as resistant in field at Baton Rouge, LA.

[e]Selected as resistant in field at Dover, FL.

[f]Selected as resistant in field at Castle Hayne, NC.

[g]From Maas, J. L., 1984. Reference number 11.

is known to enhance symptom development (14); however, previous unreported studies have shown that if leaves are cut off at least 24 hr prior to inoculation the cut surface does not serve as an infection court.

Strawberry cultivars. Strawberry cultivars were purchased as dormant crowns from a commercial nursery, potted in 10 cm plastic pots in a 1:1 (v/v) mixture of Jiffy-Mix (JPA, West Chicago, IL) and pasteurized sand, and grown for a minimum of 6 wk before inoculation. The plants were grown in a greenhouse at about 28 C with supplemental light to achieve a 16 hr photoperiod. Each plant was fertilized by placing 0.25 g of Osmocote (14-14-14) (Sierra Chemical Co., Milpitas, CA) slow release fertilizer in each pot every 8 wk beginning 6 wk after potting. Older leaves, runners, and flowers were removed from all plants 1 to 7 days before inoculation and three or four young leaves were left on at the time of inoculation.

Inoculum. C. fragariae isolates (Table 2) were grown on a 1:1 (v/v) mixture of Difco oatmeal agar and Difco potato dextrose agar in petri dishes for 7 to 14 days at room temperature (approx. 25 C) under continuous fluorescent light. Conidia were washed from the plate cultures with distilled water containing two drops of Tween 20 per liter, and the desired conidial concentration obtained by dilution and counts made with a hemacytometer. Inoculum was a 1.5×10^6 conidia/ml suspension except in the 'inoculum concentration' study in which a range of inoculum levels was tested. The inoculum was applied as a plant spray to both the leaves and petioles of the plants except in the 'inoculation methods' study wherein three other inoculation methods were also evaluated.

Table 2. Source of Colletotrichum fragariae isolates from strawberry plants with anthracnose-crown rot symptoms

Code	Isolated by	Date Isolated	Plants From
CF-1	N. Horn, La. State U.	~1968	LA
CF-4	R. Milholland, N. C. State U.	~1978	NC
CF-63	B. Smith. USDA, MS	1981	MS
CF-75	B. Smith, USDA, MS	1981	MS
CF-card	B. Smith, USDA, MS	1980	NC
CG-163	C. Howard, U. Fla.	1982	TN
CG-164	C. Howard, U. Fla.	1982	NC
FLA-2	C. Howard, U. Fla.	1978	FL
LA-1	N. Horn, La. State U.	1979	LA

Inoculation Methods. In a search for an inoculation method to use to evaluate anthracnose-crown rot resistance in strawberry plants, three separate studies were made to compare various inoculation methods. 'Study 1.' Two inoculation methods, 'plant spray' and 'plant spray plus crown drops' were compared for their effect on subsequent disease severity on 12 strawberry cultivars. 'Study 2.' Four inoculation methods were evaluated on seedling populations: 1) distal petiole spray as reported by Delp and Milholland (3); 2) plant spray, as reported by Smith and Spiers (14); 3) plant spray plus crown drops, and 4) plant spray plus crown injection. 'Study 3.' 'Crown injection' alone was used as an inoculation technique on cultivars and breeding lines. The 'distal petiole spray' inoculated plants were turned upside down and the distal half of each plant sprayed with inoculum to the point of runoff with a hand-pump sprayer. Excess inoculum was allowed to drip off prior to turning the plant upright, thereby preventing the inoculum from running into the crown. The inoculum was applied to the leaves and petioles of the 'plant spray' inoculated plants with a hand-pump sprayer to the point of runoff. No care was taken to either introduce or exclude inoculum from the crown of the plants. The 'plant spray plus crown drops' inoculated plants were spray inoculated then three drops of inoculum were placed into the crown area with a Pasteur pipette without wounding the plant. The 'plant spray plus crown injection' plants were injected with 0.2 ml of inoculum directly into the crown of each plant with a 1 cc Tuberculin syringe followed by a plant spray inoculation. The 'crown injection' plants were inoculated in the same way without the subsequent plant spray.

Immediately after inoculation regardless of the inoculation technique the plants were placed in a dew chamber to maintain free water on the foliage during the entire 48 hr incubation period and then transferred to the greenhouse for the remainder of the study. In all studies except as otherwise noted the temperature of the unlighted dew chamber was 32 $\pm$ 1 C and the greenhouse had a 16 hr photoperiod (supplemental lighting by General Electric F400 Daylight fluorescent and 40 W incandescent lights) and a temperature of about 28 C.

Dew chamber construction. The dew chamber consisted of a 0.6 m^3 (1.4m L X 0.6m W X 0.7m H) black polyethylene enclosure placed inside an unlighted growth chamber. The thermostat controlling the growth chamber was placed inside the dew chamber providing a constantly regulated air temperature inside the dew chamber. Water in a shallow pan in the bottom of the dew chamber was maintained at 5 to 7 C higher than the air temperature with an immersion heater regulated by an independent thermostat.

Disease severity rating (DSR) scale. Disease severity was rated using the method of Delp and Milholland (3) modified to include a category which recognizes symptoms of crown infection in plants still alive. The rating categories were as follows: 0 = healthy plants with no visible lesions; 1 = plants with petiole lesions <3 mm long; 2 = plants with petiole lesions 3 to 10 mm long; 3 = plants with petiole lesions 10 to 20 mm long usually girdling the petiole; 4 = plants with petiole lesions >20 mm long to the entire petiole necrotic; 5 = plant whose youngest leaf was wilted indicating a crown infection with or without petiole lesions; 6 = plant dead, crown necrotic.

Following inoculation by the various 'plant spray' methods, plants were grouped by their DSRs as follows: 0 - 2 = resistant; 2.1 - 3.9 = intermediate; and 4.0 - 6.0 = susceptible. When the 'crown injection' method of inoculation was used the plants were grouped by DSRs as follows: 0 - 3.5 = resistant; 3.6 - 4.4 = intermediate; and 4.5 - 6.0 = susceptible.

Inoculum concentration, dew chamber temperature, and greenhouse temperature. Three strawberry cultivars with varying reported reactions to anthracnose, Sequoia (resistant), Cardinal (unknown) and Tioga (very susceptible) (11), were tested for their disease reaction to two isolates of C. fragariae, CF-1 and CG-164, under various conditions using the 'plant spray' inoculation method. Four plants of each of the cultivars were used in each treatment combination. Five concentrations of inoculum (0, 7.5×10^5, 1.5×10^6, 3.0×10^6, or 6.0×10^6 conidia/ml) and three dew chamber temperatures (25, 30, and 35 C) during the first 48 hr after inoculation were tested for their effect on subsequent disease development. Following the dew chamber incubation period, plants were transferred to two greenhouses with temperatures maintained at 25 $\pm$ 3 C or 32 $\pm$ 3 C, respectively, and observed for disease development.

Seedling age. The effect of strawberry seedling age on disease severity following inoculation by C. fragariae was evaluated in two separate studies. In a 1983 study, 2-, 4-, 8-, and 14-wk-old seedlings of five selected crosses and the susceptible open-pollinated cultivar, Tufts, were divided into ten replications with seven or eight plants from each seedling population at each age. The seedlings were inoculated by the 'plant spray' method using an equal mixture of

conidial suspensions of isolates CF-1, CF-4, Fla-2, La-1, CF-75 and CF-card (1.5 X 10^6 conidia/ml of each isolate combined in equal volumes for a final concentration of 2.5 X 10^5 conidia/ml of each isolate in a total of 1.5 X 10^6 conidia/ml). In the 1984 study, two individual isolates of C. fragariae were used to inoculate seedlings. Three-, 14-, and 18-wk-old seedlings from five selected crosses and the susceptible open-pollinated cultivar, Tioga, were divided into eight groups with five plants of each seedling population at each age. Four groups (replications) at each age were inoculated with isolate CF-1 and four with isolate CG-164 by the 'plant spray' method.

Data analyses. The SAS statistical package (5) was used to conduct analysis of variance tests. If the F-test indicated significant treatment differences, mean separation was by Duncan's multiple range test, least significant difference, Waller-Duncan test, or least squares mean.

RESULTS

Inoculum concentration, dew chamber temperature, and greenhouse temperature. There were no significant differences in the overall disease severity ratings (DSRs) following inoculation with the three higher inoculum levels of isolate CF-1 or of all four inoculum levels of isolate CG-164 (Table 3). Plants inoculated with isolate CF-1 at the lowest inoculum level, 7.5 X 10^5 conidia/ml, had a significantly lower DSR than the plants inoculated with the three higher inoculum levels. Since the DSRs resulting from inoculation with the three higher inoculum concentrations were not different, the DSRs of plants in these

Table 3. Disease severity rating[a] of three strawberry cultivars 30 days after spray inoculation with two isolates of Colletotrichum fragariae at various inoculum concentrations

Isolate	Inoculum Level (Conidia/ml)	Strawberry Cultivar			Mean
		Cardinal	Sequoia	Tioga	
		— Mean DSR rating[b] —			
CF-1	0[c]	0.0	0.0	0.0	0.0
	7.5×10^5	2.8	3.2	4.3	3.4b[d]
	1.5×10^6	3.6	3.5	4.7	3.9a
	3.0×10^6	3.3	3.7	4.7	3.9a
	6.0×10^6	3.5	3.9	5.2	4.2a
		(LSD (P = 0.05) = 0.62)[e]			
CG-164	0[c]	0.0	0.0	0.0	0.0
	7.5×10^5	2.8	4.0	5.2	4.0a
	1.5×10^6	3.4	3.9	5.3	4.2a
	3.0×10^6	3.9	3.6	5.2	4.2a
	6.0×10^6	3.7	4.2	5.6	4.5a
		(LSD (P = 0.05) = 0.70)[e]			

[a]Disease severity rating (DSR) scale: 0 = no symptoms to 6 = plant dead.

[b]Average DSR of a total of 24 plants of each cultivar at each inoculum level; four plants incubated at each of three dew chamber temperatures (25, 30 and 35 C) and two greenhouse temperatures (25 and 32 C).

[c]Data for controls not included in analysis of variance.

[d]Mean separation within isolates in column by Waller-Duncan, K = 100.

[e]For cultivar-inoculum level combinations.

treatments were combined to give a total of twelve plants per treatment per isolate in the dew chamber and greenhouse studies.

There was a significant interaction between dew chamber temperatures and cultivar responses. Dew chamber temperature had no effect on the disease response of Tioga plants which were rated susceptible (DSR 4.0 - 6.0) to both C. fragariae isolates at all three dew chamber temperatures (Table 4 and Fig. 1). Cardinal plants gave progressively more severe DSRs to both fungal isolates at the higher dew chamber temperatures, resulting in an intermediate disease response at 25 and 30 C and a susceptible response at 35 C. Surprisingly, the DSRs of Sequoia plants to both fungal isolates tested were significantly lower on the plants held in the 30 C dew chamber than on plants held in the 25 or 35 C dew chambers after inoculation. There was not a significant difference among the DSRs on Sequoia plants held in the 25 or 35 C dew chambers following inoculation with either isolate of the fungus.

No interaction between the overall greenhouse temperature and cultivar response was observed. Plants of each of the three cultivars held in the 32 C greenhouse after inoculation had higher DSRs than plants of the same cultivars which were held in the 25 C greenhouse (Fig. 1 and Table 4). When DSRs were averaged over all other treatment variables, the DSR of the Tioga plants was significantly higher than the DSRs of the Sequoia and Cardinal plants. The two C. fragariae isolates responded similarly to all variables except that isolate CG-164 caused a slightly higher DSR on plants in most treatments and a higher overall DSR than isolate CF-1. The disease response curve of each cultivar to each isolate usually had little if any increase from the 30 day rating until the 50 day rating (Fig. 1).

Table 4. Effect of dew chamber and greenhouse temperatures on the 30-day disease severity rating[a] following plant spray inoculation of strawberry cultivars with Colletotrichum fragariae

Cultivar	Dew Chamber Temperature	Isolate CF-1			Isolate CG-164		
		Greenhouse Temperature (C)					
		25	32	$\bar{x}$	25	32	$\bar{x}$
		Mean disease severity rating[b]					
Cardinal	25C	2.0 h[c]	2.3 h	2.1 C[d]	2.1 f	3.3 e	2.7 B
	30C	2.8 fgh	3.9 de	3.4 B	2.6 de	3.5 de	3.0 B
	35C	4.7 bcd	5.1 abc	4.9 A	4.4 bcd	5.2 ab	4.8 A
	$\bar{x}$	3.2 B[d]	3.8 A	3.5 y[e]	3.0 B	4.0 A	3.5 y
Sequoia	25C	3.5 efg	4.2 cde	3.8 A	3.6 de	4.8 abc	4.2 A
	30C	2.6 gh	3.7 def	3.1 B	2.8 ef	3.3 e	3.1 B
	35C	4.0 de	4.3 cde	4.1 A	3.9 cde	4.8 abc	4.4 A
	$\bar{x}$	3.4 B	4.0 A	3.7 y	3.4 B	4.3 A	3.9 y
Tioga	25C	4.4 bcde	5.2 ab	4.8 A	5.1 ab	5.8 a	5.5 A
	30C	4.3 cde	5.1 ab	4.7 A	5.2 ab	5.2 ab	5.2 A
	35C	4.7 bcd	5.6 a	5.1 A	5.2 ab	5.6 a	5.4 A
	$\bar{x}$	4.5 B	5.3 A	4.9 z	5.1 A	5.5 A	5.4 z
Mean	25C	3.3	3.9	3.6 Y[e]	3.6	4.6	4.1 Y
	30C	3.2	4.2	3.7 Y	3.5	4.0	3.8 Y
	35C	4.5	5.0	4.7 Z	4.5	5.2	4.9 Z
	$\bar{x}$	3.7 Y[e]	4.4 Z	4.0	3.9 Y	4.6 Z	4.2

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Table 4. (Continued)

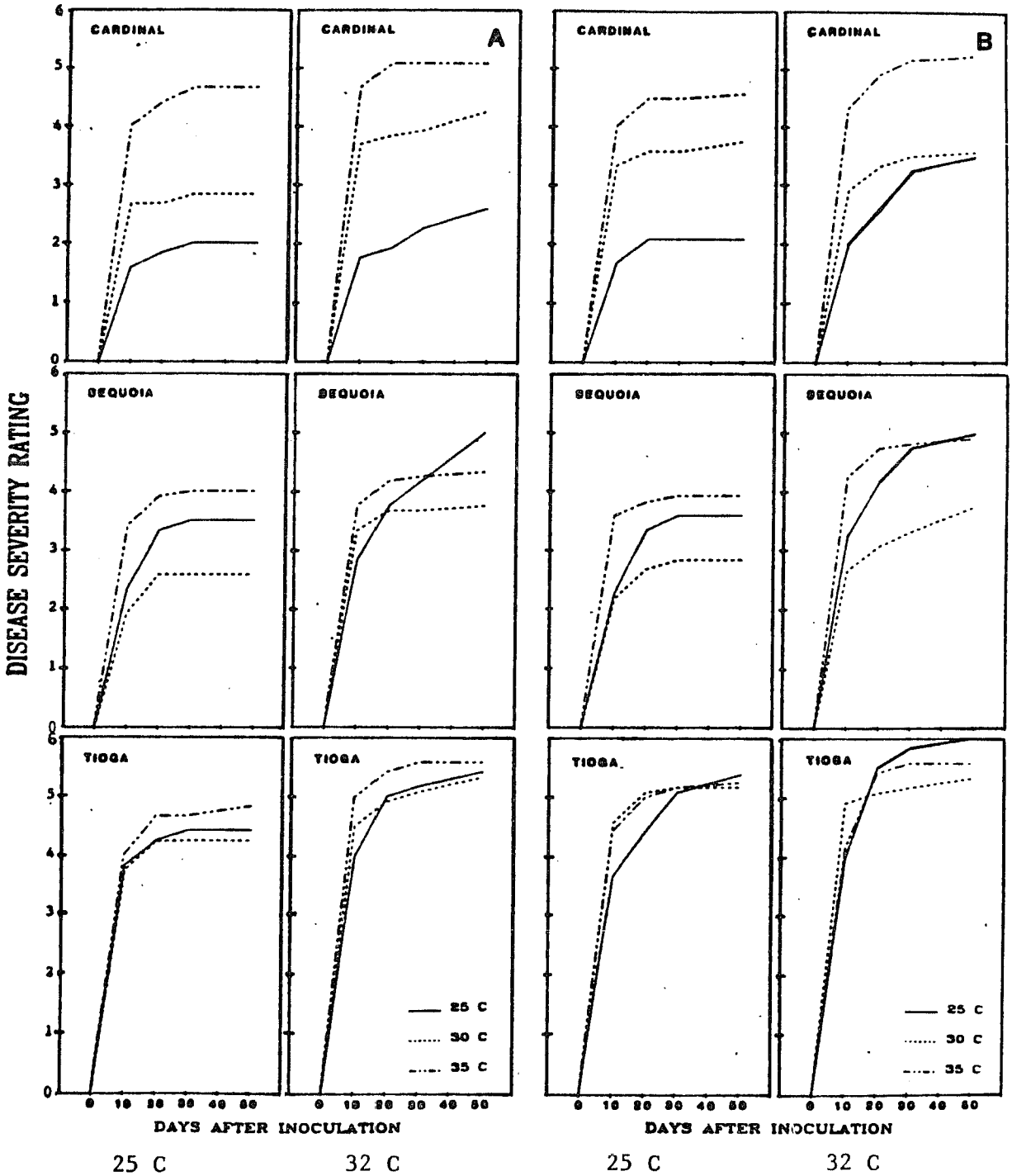
[a]Disease severity rating (DSR) scale: 0 = no symptoms to 6 = plant dead.

[b]Average DSR of 12 plants of each treatment combination (combined results from four plants at three inoculum levels, 1.5×10^6, 3.0×10^6, and 6.0×10^6 conidia/ml. There was no significant differences in the disease ratings of plants inoculated at these three levels, therefore these levels were pooled and considered as replications).

[c]Mean DSR of cultivar-dew chamber temperature-greenhouse temperature treatments separated within isolate by Duncan's Multiple Range Test; P = 0.05.

[d]DSR means due to greenhouse temperatures and dew chamber temperatures within cultivars separated by LSD = 0.05 within each isolate.

[e]Overall cultivar DSR means within each isolate and dew chamber disease severity means within each isolate separated by Waller-Duncan, K = 100.



A. C. fragariae Isolate CF-1. B. C. fragariae Isolate CG-164.

Figure 1. Disease response curve of three strawberry cultivars to plant spray inoculation with Colletotrichum fragariae isolates CF-1 (A) and CG-164 (B). Plants held in a dew chamber at 25, 30 or 35 C for 48 hr after inoculation then transferred to a greenhouse and held at 25 or 32 C for 50 days. Disease severity rating $\leq$ 2.0 = resistant, 2.1 to 3.9 = intermediate, and $\geq$ 4.0 = susceptible.

Inoculation methods. 'Study 1.' Four plants each of 12 cultivars were inoculated with five isolates of C. fragariae by the 'plant spray' method and four plants each by the 'plant spray plus crown drops' method. There were no differences in the DSRs of plants of the same cultivar inoculated by the two methods with the same isolate except for the DSRs of Titan inoculated with isolate MS-9 and Tennessee Beauty inoculated with isolate CG-164 (Table 5). There were differences in the DSRs among cultivars and among isolates.

'Study 2.' There were significant differences in the DSRs of seedling populations when four inoculation methods were compared using 8- to 14-wk-old seedlings from seven crosses of resistant strawberry lines and a single open-pollinated susceptible cultivar. Groups of six seedlings from each of the crosses were inoculated with each of two C. fragariae isolates by each of the four methods. The seedling populations reacted similarly to both isolates of C. fragariae (Table 6). In general, DSRs of seedlings inoculated by each method with isolate CG-164 were slightly higher than those inoculated by the same method with isolate CF-1. Mean DSRs for all seedling populations failed to show any differences among the 'distal petiole spray,' the 'plant spray' and the 'plant spray plus crown drops' inoculation techniques. However, the 'plant spray plus crown injection' technique resulted in a mean DSR that was significantly higher than the ratings for all the other techniques tested (Table 6). Seedlings of Tufts O.P. received DSRs of 4.7 and 5.0 following inoculation by isolates CF-1 and CG-164, respectively, by the 'plant spray plus crown injection' technique. In the same test, seedlings inoculated by this same method from populations derived from crosses of reported resistant lines had DSRs

Table 5. Disease severity ratings[a] of 12 strawberry cultivars inoculated by either a 'plant spray plus crown drops' or a 'plant spray only' method with five isolates of Colletotrichum fragariae

Strawberry Cultivar	Isolate											
	CF-4		MS-9		CF-75		CF-1		CG-164		Mean	
	Inoculation Method[b]											
	PS+ CD	PS	PS+ CD	PS	PS+ CD	PS	PS+ CD	PS	PS+ CD	PS	PS+ CD	PS
Tioga	5.2[c]	4.8	4.3	4.5	4.0	4.0	3.8	4.5	5.0	5.8	4.5	4.7
Tangi	4.5	3.8	4.5	4.3	3.3	3.8	4.0	4.0	4.0	3.5	4.1	3.9
Albritton	6.0	4.8	4.8	4.3	4.5	4.5	3.8	4.0	1.0	2.5	4.0	4.0
Sequoia	4.5	3.8	4.5	4.3	3.0	4.0	3.0	3.8	2.8	3.5	3.6	3.9
Sunrise	5.5	4.5	2.8	2.8	3.5	3.8	3.8	3.3	4.0	3.8	3.9	3.6
Florida Belle	4.5	3.8	2.8	3.3	3.0	3.8	3.3	3.3	3.3	3.3	3.4	3.5
Titan	3.8	3.5	5.3	3.3*	3.5	5.0	2.8	2.8	1.3	2.0	3.3	3.3
Tennessee Beauty	3.5	2.3	3.5	3.3	2.5	2.3	3.8	2.8	3.0	5.5*	3.3	3.2
Florida Ninety	4.0	3.8	1.5	2.0	3.5	2.5	2.5	4.3	2.8	3.5	2.9	3.2
Prelude	2.0	3.3	2.3	3.5	3.0	4.0	2.8	3.3	3.8	2.3	2.8	3.3
Apollo	2.5	3.3	3.5	2.0	3.0	2.3	1.8	2.0	2.8	2.5	2.7	2.4
Cardinal	3.0	3.0	1.5	3.0	2.0	2.3	3.0	2.5	1.5	2.0	2.2	2.6
Mean	4.1[d]	3.7	3.4	3.4	3.2	3.5	3.2	3.4	2.9	3.4	3.4[ns]	3.5[ns]

[a]Disease ratings made 30 days after inoculation on a scale of 0 = no symptoms to 6 = plant dead from anthracnose. Each rating is the average rating of four plants.

[b]PS + CD = plants inoculated by a plant spray of inoculum followed by placing three drops of inoculum directly into the crown of each plant; PS = plants inoculated by a plant spray of inoculum. Inoculum was a water suspension of 1.5×10^6 conidia per ml.

[c]LSD (0.05) = 1.88 for isolate-cultivar-method combination; * indicates mean DSRs of pair significantly different.

[d]LSD (0.05) = 0.544 for isolate-method means.

Table 6. Disease severity ratings[a] of strawberry seedlings 30 days after inoculation[b] with two isolates of Colletotrichum fragariae by various inoculation methods

		Inoculation Method[c]					
						Controls	
Isolate	Seedling Population	Distal Petiole Spray	Plant Spray	Plant Spray + Drop	Plant Spray + Inject	Plant Spray	Spray + Inject
CF-1	83-65	1.5[d]	1.4	1.3	2.5	0.0	0.1
	83-66	2.3	2.4	2.1	3.8	0.0	0.5
	83-67	1.9	1.9	1.9	2.6	0.3	1.2
	83-68	2.5	3.1	2.0	4.0	0.3	0.5
	83-72	1.5	1.5	1.5	2.5	0.0	0.0
	83-73	1.8	2.5	2.4	2.5	0.5	0.8
	83-76	2.0	1.6	1.7	3.3	0.1	0.9
	Tufts O.P.	2.2	3.0	2.1	4.7	1.2	1.1
	LSD = 0.91, P = 0.05 for host-treatment combinations						
	Mean	2.0b[e]	2.2b	1.9b	3.2a	0.3d	0.6c
CG-164	83-65	1.6	2.1	2.1	2.8	0.0	0.1
	83-66	2.7	2.5	2.5	3.5	0.0	0.5
	83-67	2.8	1.8	2.7	2.9	0.3	1.2
	83-68	2.8	3.0	2.3	3.8	0.3	0.5
	83-72	2.1	1.9	2.0	2.8	0.0	0.0
	83-73	1.8	1.9	2.6	3.8	0.5	0.8
	83-76	2.3	2.4	2.2	3.8	0.1	0.9
	Tufts O.P.	3.7	3.5	4.3	5.0	1.2	1.1
	LSD = 0.90, P = 0.05 for host-treatment combinations						
	Mean	2.5b[e]	2.4b	2.6b	3.6a	0.3d	0.6c

[a]Disease severity rating scale; 0 = no symptoms to 6 = plant dead.

[b]Inoculated at 8 - 14 wk of age after the first-true-leaf stage.

[c]Inoculation methods described in text.

[d]Average disease severity rating of four replications of six plants per replication for each seedling population - treatment combination.

[e]Means followed by the same letter within a row are not significantly different according to Waller-Duncan, K = 100.

that ranged from 2.5 to 4.0. Fifty days after inoculation, 65 to 98% of the seedlings of the seven resistant populations inoculated by the 'plant spray plus crown injection' method were still surviving, but only 32% of the Tufts O.P. seedlings remained alive (Table 7).

'Study 3.' The results of the preceeding study indicated that some plants are resistant to crown infection and they can be identified by crown injection. Therefore, a 'crown injection' inoculation without any foliar inoculation was used to evaluate resistance to crown infection of three breeding lines previously selected as anthracnose-crown rot resistant and four standard cultivars. Four plants of each line and cultivar were injected with inoculum from each of six C. fragariae isolates. After incubation in the dew chamber, the plants were transferred to a greenhouse with a temperature of about 32 C. Thirty days after crown injection with most of the isolates most of the cultivars received DSRs $\geq$ 4.5; whereas, two of the lines, MSUS 42 and MSUS 70 received DSRs of $\leq$ 4.0 to most of the isolates (Table 8). Fifty days after inoculation only three of the MSUS 42 plants injected with inoculum of the six isolates had died and only nine of the MSUS 70 plants out of a total of 24 plants of each line had died. By comparison, of the 24 inoculated plants of each of the cultivars, 15 to 22 plants of each cultivar were dead 50 days after inoculation. Many of the plants inoculated by crown injection received DSRs of less than "5", indicating a petiole symptom rather than a crown symptom. These plants usually had a lesion on the petiole of a young leaf which had not yet emerged from the crown at the time of inoculation.

Seedling age. The younger seedlings, in general, received significantly higher DSRs than older seedlings in both the 1983 and

Table 7. Survival of strawberry seedlings inoculated[a] by 'plant spray plus crown injection' method 50 days after inoculation with isolates CF-1 or CG-164 of Colletotrichum fragariae

Seedling Population	C. fragariae Isolate		
	CF-1	CG-164	Mean
	Percentage of Survival[b]		
83-65	83	96	90
83-66	87	88	88
83-67	100	96	98
83-68	75	54	65
83-72	96	100	98
83-73	100	83	92
83-76	75	63	69
Tufts O.P.	32	32	32

[a]Inoculated at 8 - 14 wk after the first-true-leaf stage.

[b]Based on 24 seedlings; six per replication with four replications.

Table 8. Mean disease severity ratings[a] 30 days after inoculation and the number of plants dead 50 days after inoculation of strawberry cultivars and lines inoculated by crown injection with a conidial suspension of six Colletotrichum fragariae isolates

	Strawberry cultivar or line													
	Rosanne		Tangi		MSUS 27		TN Beauty		Cardinal		MSUS 70		MSUS 42	
Isolate	DSR	No. Dead	DSR	No. Dead	DSR	No. Dead	DSR	No. Dead	DSR	No. Dead	DSR	No. Dead	DSR	No. Dead
CG-164	4.8[b]	2	6.0	4	6.0	4	6.0	4	3.3	1	4.0	3	4.3	2
CF-card	6.0	4	4.8	3	5.8	4	5.3	3	5.3	3	2.8	1	3.5	0
CG-163	5.3	4	5.5	4	5.3	3	4.0	2	5.3	4	4.8	2	3.3	0
Fla-2	6.0	4	5.5	3	5.5	3	4.3	3	4.0	2	2.8	0	4.0	1
CF-63	6.0	4	5.5	4	4.3	2	3.3	2	4.8	3	3.8	2	3.0	0
CF-1	6.0	4	6.0	4	3.5	1	5.0	3	4.0	2	3.5	1	2.5	0
Mean	5.7	3.7	5.5	3.7	5.1	2.8	4.6	2.8	4.4	2.5	3.6	1.5	3.4	0.5
Water[c]	0.0	0	0.8	0	0.3	0	0.8	0	2.0	0	0.0	1	0.0	0

[a]Disease severity rating (DSR) scale: 0 = no symptoms to 6 = plant dead.

[b]LSD (0.05) within cultivar-isolate means = 1.98 for crown injected plants.

[c]Plants injected with sterile distilled water.

1984 studies (Tables 9 and 10). In the 1983 study, the overall average DSRs of the 14-wk-old seedlings were significantly lower than the ratings of the three younger ages tested, although there were no significant differences in the ratings within four of the individual seedling populations between the 8- and 14-wk-old seedlings (Table 9). No seedling population had a mean DSR of $\leq$ 2.0 when inoculated at the 2- or 4-wk-old stages, but three seedling populations had DSRs $\leq$ 2.0 (resistant to 'plant spray' inoculation) when inoculated at the 14-wk-old stage. Again in the 1984 experiment, older inoculated seedlings were found to be the most resistant to each isolate of C. fragariae (Table 10). Seedling populations inoculated with isolate CF-1 at the 3-wk-old stage received a significantly higher overall average DSR than 14-wk-old plants which in turn received a significantly higher overall DSR than 18-wk-old plants. Seedling populations inoculated at the 3-wk-old stage with isolate CG-164 received a significantly higher overall average DSR than seedlings inoculated at the 14- and 18-wk-old stages. But, there was no significant difference between the overall average DSRs of the 14- and 18-wk-old plants inoculated with this isolate.

Resistance to C. fragariae in strawberry seedling populations. Most of the seedling populations from crosses among selected strawberry lines and cultivars were found to possess a greater level of resistance to isolates of C. fragariae than the Tioga O.P. or Tufts O.P. seedlings. Several of the seedling populations demonstrated a high level of resistance. For example, from the 1983 seedling age study, populations 82-79, 82-80, and 82-85 had DSRs $\leq$ 2.0 following inoculation at 14 wk of age (Table 9). From the 1984 studies seedling

Table 9. 1983 disease severity ratings[a] of strawberry seedlings 5 wk after plant spray inoculation with a mixture of six Colletotrichum fragariae isolates; CF-1, CF-4, FLA-2, LA-1, CF-75 and CF-card

Seedling Population	Seedling Age (wk)[b] 2	4	8	14	Mean
	Mean Disease Severity Rating[c]				
82-79	2.4 ab(y)[d]	3.3 a(y)	1.5 ab(x)	1.2 b(x)	2.1 w
82-80	2.9 ab(y)	3.3 a(y)	2.5 ab(x)	1.1 b(x)	2.4 wx
82-85	4.0 a (yz)	4.2 a(yz)	3.7 a (yz)	1.4 b(xy)	3.3 xy
82-78	5.6 a (z)	4.9 a(yz)	3.3 ab(xy)	2.1 b(xyz)	4.0 y
82-84	5.9 a (z)	5.9 a(z)	5.3 ab(yz)	3.8 b(z)	5.2 z
Tufts O.P.	5.9 a (z)	6.0 a(z)	5.5 a (z)	3.2 b(yz)	5.2 z
Mean	4.4 ab	4.6 a	3.6 b	2.1 c	

[a]Disease severity rating scale; 0 = no symptoms to 6 = plant dead.

[b]Age of seedlings at the time of inoculation based on time after transplanting at the first-true-leaf stage.

[c]Average rating of seven or eight seedlings per replication and 10 replications per age group.

[d]Mean separation in rows (a - c) and mean separation in column (w - z) by Least Squares Mean, P = 0.05.

Table 10. 1984 disease severity ratings[a] of strawberry seedlings 30 days after plant spray inoculation by Colletotrichum fragariae isolates CF-1 and CG-164

Seedling Population	Seedling Age (wk)[b] 3	14	18
	Average disease severity rating[c]		
	Isolate CF-1		
83-65	2.2[d]	2.4	2.2
83-66	4.1	2.3	2.3
83-70	5.4	3.0	2.2
83-73	5.1	2.6	1.9
83-76	5.3	3.8	3.3
Tioga O.P.	5.9	4.6	3.7
Average	4.7a[e]	3.1b	2.6c
	Isolate CG-164		
83-65	4.3[f]	2.3	2.3
83-66	5.1	2.2	3.5
83-70	5.9	3.0	2.9
83-73	5.9	2.8	2.0
83-76	6.0	4.1	3.7
Tioga O.P.	5.9	4.4	5.2
Average	5.5a[e]	3.1b	3.3b

[a]Disease severity rating (DSR) scale: 0 = no symptoms to 6 = plant dead.

[b]Age of seedlings at the time of inoculation based on time after transplanting at the first-true-leaf stage.

[c]Average DSR of five plants per replication and four replications per age.

[d]LSD = 1.31 for each seedling population-age combination, P = 0.05.

[e]Mean separation in row by Waller-Duncan, K = 100.

[f]LSD = 1.03 for each seedling population-age combination, P = 0.05.

populations, 83-65, 83-67, 83-72, and 83-73, consistently had low DSRs following inoculation of older seedlings (Tables 6 and 10) and had a high percentage of survival following 'crown injection' inoculation (Table 7). The fact that three of the seedling populations (82-79, 82-80 and 83-65) had low DSRs at all inoculation ages suggests that they possess juvenile as well as mature plant resistance.

DISCUSSION

Inoculum concentrations of less than 10^6 conidia/ml of C. fragariae may not be adequate for a reliable evaluation of the anthracnose-crown rot response by strawberry plants. However, concentrations ranging from 1.5×10^6 to 6.0×10^6 conidia/ml were equally effective, that is, the higher inoculum levels did not result in significantly higher DSRs.

Plants held at higher temperatures (32 C) after inoculation developed more severe disease symptoms than those held at lower temperatures (25 C). However, the temperature within the dew chamber during the initial 48 hr incubation period had more influence on disease severity ratings (DSRs) of some cultivars than did the later greenhouse incubation temperatures. Plants incubated at the highest dew chamber temperature (35 C) in general received higher DSRs than comparable plants held at 25 or 30 C, but the level of effect of dew chamber temperature was very different among cultivars (Table 4 and Fig. 1). For example, the DSR for Cardinal was almost doubled when the plants were incubated in the 35 C dew chamber rather than at 25 C. The difference in DSRs at these temperatures were great enough to change this cultivar's rating from near resistant to very susceptible. On the

other hand, Tioga plants responded similarly to all dew chamber temperatures (25, 30 and 35 C) and at both greenhouse temperatures (25 and 32 C). This cultivar would be rated susceptible at any combination of the enviromental conditions used in this experiment. Sequoia plants anthracnose-crown rot response ranged from intermediate to susceptible in these tests. Sequoia plants held in the 25 C dew chamber had higher DSRs than those held at 30 C and those held at 35 C had higher DSRs than either. Enviromental conditions play an important role in the development of anthracnose-crown rot, making controlled, defined environmental conditions necessary when evaluating plant response to this disease. The independent way in which cultivars responded to different dew chamber temperatures, i.e., the temperature and high humidity during the initial stages of infection, may explain in part why cultivars may appear resistant in one location and not another (4, 11).

Age is another important consideration when screening strawberry seedlings for resistance to C. fragariae. In general, seedlings become more resistant as they increase in age up to 14 wk (Tables 9 and 10); and, resistance in some seedling populations can be identified only after seedlings attain this age. Two-wk-old seedling from three populations in this study expressed a distinguishable level of resistance suggesting that resistant selections could be made at an early age, but if this is done some sources of resistance that are not expressed until a later developmental stage may be eliminated.

Three of the inoculation methods, 'distal petiole spray,' 'plant spray' and 'plant spray plus crown drops,' resulted in DSRs for anthracnose-crown rot that were not significantly different from each

other on the seedling populations or clonal lines. While any of these three methods could be used to evaluate plant response to the petiole phase of anthracnose-crown rot, the 'plant spray' method would be easiest to use and as reliable as the other two.

One of our primary objectives was to identify plants resistant to the crown rot phase of anthracnose-crown rot. Therefore, it appeared necessary to use an inoculation method which introduced the inoculum into the crown of the plant. To do this the 'plant spray plus crown injection' and the "crown injection" methods were used. The seedling populations and breeding lines chosen for this study were derived from parental lines that were designated anthracnose-crown rot resistant based on field observations and/or greenhouse spray inoculation tests (Table 1). A high level of resistance to the crown rot phase of the disease was demonstrated by the survival of many or all of the plants of several seedling populations (Table 7) and two breeding lines (Table 8) following crown injection with conidia from several individual C. fragariae isolates. The crown injected plants invariably had DSRs higher than spray inoculated plants when the same fungal isolates were compared (Tables 5, 6, and 8). But, when adjustments were made to the disease response ranges to reflect the severity of the inoculation methods, most cultivars and seedling populations responded similarly. The original and adjusted DSR ranges are as follow: after plant spray inoculation DSR $\leq$ 2.0 = resistant, 2.1 to 3.9 = intermediate, $\geq$ 4.0 = susceptible; after crown injection DSR $\leq$ 3.5 = resistant, 3.6 to 4.4 = intermediate, $\geq$ 4.5 = susceptible.

The results of these studies clearly show that crown injection does not overcome high levels of resistance to anthracnose-crown rot. In

fact, crown injection may be the best inoculation method to use when screening advanced resistant selections, because this rigorous method will kill and thus eliminate the less resistant plants from the program. This method also guards against the potential selection of strawberry lines that have foliar anthracnose-crown rot resistance but no crown resistance. In addition any line resistant to crown infection was also resistant to the foliar phase of anthracnose-crown rot, thus making a plant spray inoculation unnecessary. The DSR scale used in these studies is based primarily on petiole reponse and was used to provide a direct comparison of disease response of plants inoculated by the various methods; but, this DSR scale would not be the best rating scale to use to evaluate plants inoculated by a crown injection. For these plants, a simple 'alive' or 'dead' rating could be made 50 days after inoculation.

The disease response of the progeny from seedling populations in the current study cannot be compared directly with the reported response of their parent lines because the parental response was usually based on field observations (Table 1). However, most seedling populations which had a parent that had been rated either very resistant (VR) or which was a selection from the anthracnose screening program (R_2) were found to be more resistant than progeny of parents that had been rated resistant (R) or susceptible (S). These results suggest that a breeding program coupled with a good disease resistance screening program could lead to the development of strawberry cultivars resistant to anthracnose-crown rot.

No attempt was made in this study to identify pathogenic races of C. fragariae. However, the variation in disease response among

strawberries of different origin to individual fungal isolates and among different isolates to individual strawberry lines or cultivars strongly suggest that races occur. Therefore, until these details are worked out, a screening program for anthracnose-crown rot resistance should employ several isolates of C. fragariae to minimize the chance of selecting a very narrow genetic resistance base.

To assess the level of resistance to anthracnose-crown rot in strawberry plants, inoculation methods and environmental conditions that are conducive to severe disease development should be used. The results of this study suggest that 1) the inoculum level should be approximately 10^6 conidia/ml, 2) inoculum may be applied as either a plant spray or injected into the crown, 3) following inoculation the plants should be held in a chamber with 100% RH at 32 to 35 C for 48 hr, and 4) after removal from the high humidity chamber the plants should be maintained under good growing conditions at 25 to 32 C during the remainder of the study to allow for disease development and symptom expression. Strawberry seedlings being screened for resistance should not be inoculated until they are $\geq$14 wk beyond the first-true-leaf stage unless the objective is to limit the search for anthracnose-crown rot resistance to juvenile sources.

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Chapter II

MORPHOLOGICAL, CULTURAL AND PATHOGENIC VARIATION AMONG STRAWBERRY ISOLATES OF *COLLETOTRICHUM FRAGARIAE* AND *C. ACUTATUM*

MORPHOLOGICAL, CULTURAL AND PATHOGENIC VARIATION AMONG STRAWBERRY ISOLATES OF *COLLETOTRICHUM FRAGARIAE* AND *C. ACUTATUM*

Colletotrichum fragariae Brooks was described as a new species and identified as the causal organism of strawberry anthracnose (referred to herein as anthracnose-crown rot) by Brooks in Florida in 1931 (2). The disease was characterized as having black, sunken lesions on the stolons. Since 1931, there have been additional reports in which *C. fragariae* has been shown to cause petiole lesions (4), crown infections (3, 12), fruit rots (15), and leaf spots (16, 18) of strawberry. The crown rot phase of anthracnose-crown rot is often devastating in the southeastern United States in fruit production fields during early spring following the use of infected transplants in the fall and also in nurseries during the summer months due to the rapid spread of the pathogen through fields during warm, humid weather (12, 14, 20). The reported geographic distribution of *C. fragariae* now includes Louisiana (6), Arkansas (34), North Carolina (21), Oklahoma, and Tennessee (20) within the United States and abroad in Argentina (26), Brazil (5), Mexico (25) and India (31).

The morphological characteristics of *C. fragariae* isolates in most reports (6, 14, 15, 18, 19) have been similar to those stated in Brooks' original decription (2). However, there has been some uncertainty expressed about the correct name for the fungus. Von Arx (39) considered *C. fragariae* synonomous with *C. gloeosporioides* Penz.

(anamorph of Glomerella cingulata (Stonem.) Spauld, & Schrenk). Howard and Albregts (17, 18) have used the designation, C. gloeosporioides (=C. fragariae), but they more recently have concluded (20) that "either two distinct pathogenic species or two pathotypes of a single species are involved in this disease." In this regard, they have retained the name C. fragariae for isolates from Florida-grown strawberry plants that have never been observed to produce an ascigerous stage and use C. gloeosporioides (=G. cingulata) for isolates that produce the ascigerous stage in culture and which for the most part have been isolated from strawberry plants obtained from nurseries in Arkansas, North Carolina, and Tennessee (19, 20).

Fungi other than C. fragariae also have been reported to cause anthracnose diseases of strawberry. These include C. acutatum Simmonds (30), C. dematium (Pers. ex Fr.) Grove (1), and Gloeosporium spp. (22, 35, 36); however, none of these fungi have been reported to cause the crown rot symptoms associated with anthracnose-crown rot. Gloeosporium spp. were reported to cause a ripe fruit rot as well as stolon, petiole, peduncle and pedicel lesions of strawberries in Australia (35, 36); a fruit rot of strawberries shipped from Louisiana to Chicago (40); and a fruit rot in fields in Maryland (22). Subsequently, Simmonds (30) included the Australian Gloeosporium sp. within a newly established species, C. acutatum, whose fusiform conidia distinguished it from other Colletotrichum species with straight conidia (37). C. acutatum also causes fruit rots on a wide range of hosts and recently was reported causing petiole and runner lesions as well as a fruit rot on strawberry plants in England which had been obtained from California (7).

Colletotrichum dematium (1) was reported to cause fruit rot and petiole lesions on plants in Michigan, but did not cause crown rot. It is easily distinguished from C. fragariae and C. acutatum by its sickle-shaped conidia, and was reported (1) to be weakly pathogenic when compared to C. fragariae on strawberry fruit.

In the development of a screening program (33) to identify anthranose-crown rot resistant plants, several Colletotrichum isolates from strawberry plants were collected locally and from various locations across the United States. It was apparent that some isolates in the collection probably were not C. fragariae due to considerable variation in the cultural and morphological characteristics among the isolates obtained. Although most of these atypical isolates were obtained from fruit with anthracnose fruit rot symptoms, some were isolated from the crown of plants wilting and dying in fields in which anthracnose fruit rot was severe. A part of the present study was initiated to characterize the variations among these isolates and to determine if some of the isolates were species other than C. fragariae.

In his early work on anthracnose-crown rot, Brooks (2, 3, 4) did not report the cultivars on which this disease was occurring in Florida or if susceptibility varied among cultivars being grown at that time. Since then, however, there have been reports of variation among strawberry breeding lines and cultivars in their degree of susceptibility to C. fragariae. Horn et al. (13) separated eight isolates of C. fragariae into three races using six breeding lines and two cultivars of strawberries as indicators. Delp and Milholland (9) found a wide range of disease responses among 16 cultivars and three breeding lines to infection by ten isolates of C. fragariae, but did

not attempt to separate the isolates into races. Two of the cultivars, Dover and Sequoia, and two of the breeding lines were resistant; seven cultivars were susceptible, and the remaining seven cultivars and one breeding line were intermediate in their average anthracnose reaction to the ten isolates tested. A portion of the present study (Chapter 1) showed cultivars and breeding lines to vary in their disease response to several C. fragariae isolates.

Field observations of anthracnose-crown rot severity on strawberry cultivars in yield trials at Poplarville, MS have varied from year to year and do not always agree with the disease response published for these cultivars (Table 1) (32). These observations led to speculation that forms of C. fragariae occur at Poplarville that differ from those in other areas in their virulence to various strawberry cultivars or that different enviromental conditions result in an altered disease response. A part of the present study was initiated a) to examine the disease responses of several strawberry lines and cultivars inoculated with various isolates of C. fragariae and C. acutatum, and b) to use this information to select appropriate isolates of C. fragariae to use in the anthracnose-crown rot resistance breeding program.

MATERIALS AND METHODS

Isolates. Twenty-four Colletotrichum isolates were collected over a period of 16 years from the southeastern United States and from California (Table 2). The isolates were maintained on agar slants with periodic inoculation onto and reisolation from susceptible strawberry

Table 1. Strawberry cultivars and lines utilized in this study and their reported responses to Colletotrichum fragariae

Strawberry line	Origin	Reported disease response[a]		
		Field Observations		Greenhouse
		Published[b]	USDA-MS[c]	Test
Albritton	North Carolina	VS	-	S[d]
Apollo	North Carolina	I	I	I[d]
Cardinal	Arkansas	-	I	-
Florida Belle	Florida	R	S	-
Florida 90	Florida	-	I	I[d]
MSUS 27	Mississippi	-	R	R[e]
MSUS 42	Mississippi	-	R	R[e]
MSUS 70	Mississippi	-	R	R[e]
Prelude	North Carolina	S	-	S[d]
Rosanne	North Carolina	R	-	I[d]
Sequoia	California	R	R	R[d]
Sunrise	Maryland	S	I	S[d]
Surecrop	Maryland	VS	-	-
Tangi	Louisiana	S	-	-
Tenn. Beauty	Tennessee	VS	-	I[d]
Tioga	California	VS	S	-
Titan	North Carolina	VS	-	I[d]

[a]VS = very susceptible; S = susceptible; I = intermediate; R = resistant; - = unknown.

[b]From Mass, J. L. 1984. Reference number 22 (Table 2).

[c]Unpublished data from 1981-82 strawberry yield trials at USDA-ARS Small Fruit Research Station, Poplarville, MS.

[d]From Delp, B. R., and R. D. Milholland. 1981. Reference number 9.

[e]Anthracnose resistant selection from USDA-ARS greenhouse screening and field testing at Poplarville, MS.

Table 2. Source and designation of Colletotrichum isolates obtained from strawberry plants

Isolate Designation	Isolated by, at	Origin[a]	Isolation From	Year Isolated
CF-1	N. Horn, La. St. U.	LA	Crown	~ 1968
CF-4	R. Milholland, N. C. St. U.	NC	Crown	~ 1978
Fla-1	C. Howard, U. Fla.	FL	Crown	1978
Fla-2	C. Howard, U. Fla.	FL	Crown	1978
MS-9	B. Smith, USDA, Miss.	MS	Crown	1978
La-1	N. Horn, La. St. U.	LA	Crown	1979
La-2	N. Horn, La. St. U.	LA	Crown	1979
CF-card	B. Smith, USDA, Miss.	MS (NC)[b]	Crown	1980
CF-56	B. Smith, USDA, Miss.	MS	Crown	1981
CF-63	B. Smith, USDA, Miss.	MS	Crown	1981
CF-75	B. Smith, USDA, Miss.	MS	Crown	1981
Ark C-1	R. Sterne, U. Ark.	AK	Crown	1982
Ark P-1	R. Sterne, U. Ark.	AK	Petiole	1982
CF-167	C. Howard, U. Fla.	FL (AK)	Fruit	1982
CG-162	C. Howard, U. Fla.	FL	Crown	1982
CG-163	C. Howard, U. Fla.	FL (TN)	Crown	1982
CG-164	C. Howard, U. Fla.	FL (NC)	Crown	1982
Goff	W. Goff, S. W. MO St. U.	MO (AK)	Petiole	1982
Mil-1	B. Smith, USDA, Miss.	MS (AK)	Crown	1983
Mil-2	B. Smith, USDA, Miss.	MS (AK)	Fruit	1983
Cal A	S. Wilhelm, U. Cal.	CA	Fruit	1984
Cal B	S. Wilhelm, U. Cal.	CA	Fruit	1984
Cal C	S. Wilhelm, U. Cal.	CA	Fruit	1984
Cal D	S. Wilhelm, U. Cal.	CA	Fruit	1984

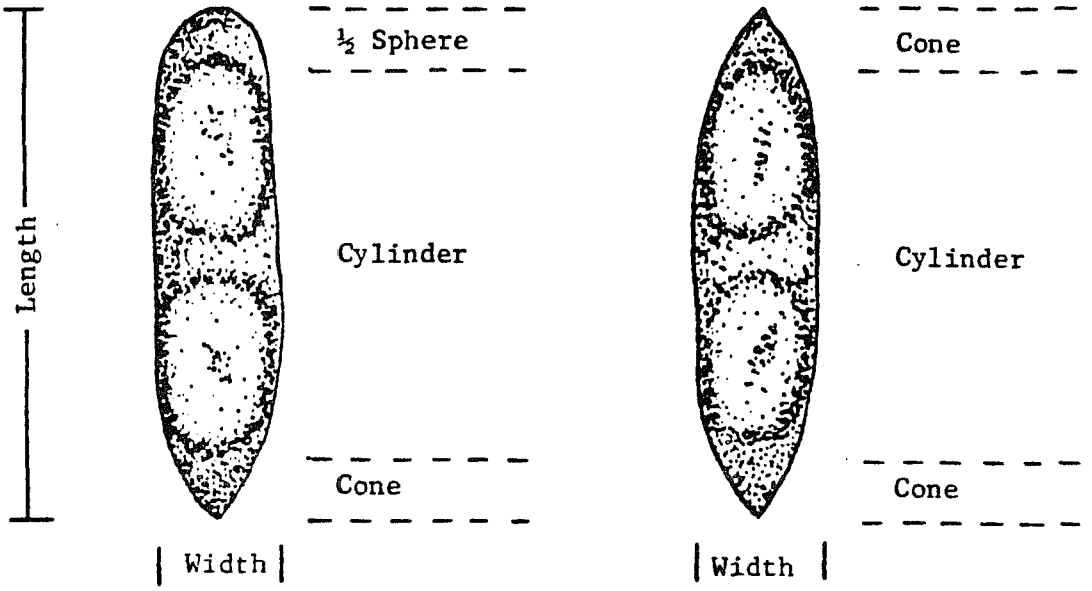
[a]Location of production field or nursery in which the plant was growing at the time isolate was obtained.

[b]Location of nursery from which the plants originated if known and different from the production field.

plants to reselect for pathogenicity from the time of their receipt until June 1983 when single-spored cultures were derived from each isolate and stored on silica gel (29). The silica gel cultures were stored at 4 C and a fresh agar culture of each isolate was started prior to the beginning of each study.

Conidial characteristics. Each isolate was grown on Difco potato dextrose agar (PDA) for 9 to 12 days under continuous fluorescent light at room temperature (approx. 25 C). A conidial suspension was prepared in sterile distilled water and conidial shape determined by examining at least 100 randomly chosen conidia of each isolate and placing each in one of three shape categories: 1) fusiform, cylindrical conidia pointed on both ends (Fig. 1b); 2) tapered cylindrical, those tapered to a point on one end and rounded on the other (Fig. 1a); and 3) cylindrical, those rounded on both ends. Each isolate was then placed in a shape catergory representive of the shape of the majority of the conidia. The length and width of 25 randomly chosen conidia of each isolate were measured, the measurements repeated four times with different cultures of the same isolate and expressed as a range of means. To provide a method of comparing the total size of the conidia among isolates within a species, the total size of each conidium was calculated as the volume (V) from the length (ℓ) and width (w) measurements. The volume of the C. fragariae conidia was calculated by the formula $V = 0.25\pi w^2 (\ell - 0.5\ w)$ which combines the volumes of a cone, a cylinder and 1/2 a sphere, roughly the shape of the C. fragariae conidia (Fig. 1a). The volume of the C. acutatum conidia was calculated by the formula $V = 0.25\pi w^2 (\ell - 0.67\ w)$. This formula

A. C. fragariae conidium B. C. acutatum conidium



If, Length = ℓ, width = w, and volume = V;

And, $V_{cylinder} = \pi r^2 h$, with $r = \frac{1}{2}w$ and $h = \ell - w$,

$V_{cone} = \frac{\pi r^2 h}{3}$, with $r = \frac{1}{2}w$ and $h = \frac{1}{2}w$,

$V_{\frac{1}{2}\ sphere} = \frac{4\pi r^3}{2 \cdot 3}$, with $r = \frac{1}{2}w$;

Then, for conidia of C. fragariae,

$$V_{Total} = V_{cylinder} + V_{cone} + V_{\frac{1}{2}\ sphere}.$$

$$V_{Total} = 0.25\pi w^2 \ (\ell - 0.5w).$$

While, for conidia of C. acutatum,

$$V_{Total} = V_{cylinder} + V_{cone} + V_{cone}.$$

$$V_{Total} = 0.25\pi w^2 \ (\ell - 0.67w).$$

Figure 1. Derivation of formulas used to calculate the volumes of C. fragariae (A) and C. acutatum (B) conidia.

combines the volumes of a cylinder and two cones which approximates the shape of the C. acutatum conidium (Fig. 1b).

The color of the conidia of each isolate was determined by scraping conidia from a 14-day-old culture grown on a 1:1 (v/v) mixture of Difco PDA and Difco oatmeal agar (PDA:OMA) under continuous fluorescent light at room temperature and examining the color against a white ceramic background.

Appressorial characteristics. Appressoria were studied using a slide culture method modified from Hawksworth (10). Mycelial and conidial water agar slide cultures were made of each of the isolates by placing a drop of cooled, molten water agar on a sterilized slide and inoculating the droplet with a mycelial tip or a drop of conidial suspension. A sterile cover slip was placed over the inoculated water agar droplet and the slide culture held in a petri dish moisture chamber for 6 to 8 days, after which appressorial formation against the cover slip was examined with the cover slip left in place.

Setae Production. Setae production in culture was determined by examining 7- to 17-day-old-cultures of each isolate grown under continuous fluorescent light on either PDA or PDA:OMA. Presence or absence of setae in culture were confirmed by examination of colonies with a stereoscope and mounts of each with the compound microscope. Setae production on the host was determined by examining strips of epidermis from lesions formed on the petiole 10 to 20 days after wound inoculation of Albritton, Tangi, or Tioga strawberry cultivars with 1.5 X 10^6 conidia/ml of inoculum from each isolate. If setae were present, ten randomly chosen setae from different acervuli on Tioga were measured.

Cultural characteristics. Each isolate was grown for 8 days on PDA under alternating near UV light (12 hr) and dark (12 hr), and the culture then examined against a white background to determine color. The relative growth rates of the isolates were determined by flooding the surface of acidified PDA (1 ml lactic acid/liter) in petri dishes with one ml of a conidial suspension containing 6000 conidia/ml. The inoculum was uniformly distributed over the agar surface, and the plates were maintained for 5 days under continuous fluorescent light at room temperature. Amount of growth was rated by two independent observers on a visual scale in which 0 = no growth and 10 = 100% of agar surface covered by mycelial growth.

Inoculum and plant spray inoculation. Isolates were grown on PDA:OMA in petri dishes for 7 to 14 days at room temperature (approx. 25 C) under continuous fluorescent light. Inoculum concentration was adjusted using a hemacytometer to 1.5 X 10^6 conidia/ml in sterile, distilled water containing two drops of Tween 20/liter. Unless noted otherwise, the inoculum was applied as a plant spray to the above ground plant parts with a hand pump sprayer by misting until the inoculum began to run off. Immediately after inoculation, the plants were held in a dew chamber with near 100% RH at 32 $\pm$ 1 C for 48 hr, and then returned to the greenhouse with the same conditions in which they were propagated prior to inoculation.

Strawberry plants. Strawberry cultivars except Tangi (Table 1) were purchased as dormant crowns from commercial nurseries, potted in 10 cm pots in a 1:1 (v/v) mixture of Jiffy-Mix (JPA, West Chicago, IL) and pasteurized sand and grown for a minimum of 6 wk before inoculation. Plants of numbered lines and Tangi were propagated from

stock mother plants in the greenhouse and grown for a minimum of 12 wk before inoculation. The plants were grown in a greenhouse at about 28 C with supplemental lights (General Electric F400 Daylight flourescent and 40 W incandescent) to achieve a 16 hr photoperiod and were fertilized with 0.25 g of Osmocote (14-14-14) (Sierra Chemical Co., Milpitas, CA) per pot every 8 wk beginning 6 wk after potting. Older leaves, runners and flowers were removed from all plants 1 to 7 days before inoculation leaving three or four young leaves on each plant at the time of inoculation.

Tissue susceptiblity. The capability of each of the 24 isolates to cause a fruit rot was tested using fruit from a flat of strawberries from Plant City, FL purchased at a local supermarket. Unblemished fruit were surface sterilized by immersing in 95% ethyl alcohol for 1 min, 0.525% sodium hypochlorite solution for 20 minutes, and rinsing in sterile distilled water four times followed by air drying (15). Four fruit were placed individually in 100 ml sterilized beakers and inoculated with each fungal isolate by placing one drop of inoculum on the side of each fruit. Controls consisted of 12 fruit handled similarly but treated with a sterile, distilled water drop. The beakers containing the inoculated fruit were held in closed plastic refrigerator boxes containing moistened paper towels for 5 days at room temperature under continuous fluorescent light. Fruit rot development was rated as postive if a firm, tan rot developed at the inoculation site on the fruit. Isolations were made from at least two of the lesions caused by each isolate to confirm the identity of the fruit rotting agent.

The capability of each of the 24 isolates to infect Tangi and Tioga strawberry plants was tested by wound inoculation of leaves and petioles of two plants of each cultivar by pricking through an inoculum drop with an insect pin. The plants were placed in a dew chamber for 48 hr after inoculation and disease responses were recorded 10 and 15 days later. A leaf spot at least 3 mm in diameter and a petiole lesion at least 3 mm long were considered positive responses to individual tests.

Cultivar-isolate interaction after plant spray and crown injection inoculations. A preliminary study conducted in 1983 using 16 strawberry lines as hosts indicated that there was pathogenic variation among the 12 Colletotrichum isolates tested (unpublished results). Results from the 1983 study were the basis of the selection of the 15 host lines used in the plant spray inoculation portion of the current study. These host lines included two cultivars susceptible to anthracnose-crown rot, Surecrop and Tioga, and a resistant breeding line, MSUS 70. The other lines are commercical cultivars whose anthracnose-crown rot reactions vary from susceptible to resistant (Table 1). Four plants of each line or cultivar were each inoculated with one of 20 isolates of Colletotrichum.

Pathogenic variation among six C. fragariae and two C. acutatum isolates was evaluated on four strawberry cultivars and three breeding lines by injecting 0.2 ml of a 1.5×10^6 conidia/ml suspension from each isolate into the crown of the plants with a Tuberculin syringe. The needle of the syringe was inserted about 5 mm deep into the plant crown at a 45° angle at the base of the fourth youngest leaf. Two sets of control plants were included in the study: one set was injected with sterile, distilled water; the other set was not injected.

Disease severity rating (DSR) scales. Plants inoculated by the plant spray method were rated for severity of disease expression 10, 20, 30 and 50 days after inoculation on a scale modified from that of Delp and Milholland (8) by adding an additional category which recognized a crown infection in plants still alive. The DSR categories were as follow: 0 = healthy plants with no visible lesions; 1 = plants with petiole lesions <3 mm long; 2 = plants with petiole lesions 3 to 10 mm long; 3 = plants with petiole lesions 10 to 20 mm long usually girdling the petiole; 4 = plants with petiole lesions >20 mm long to the entire petiole necrotic; 5 = plant whose youngest leaf was wilted indicating a crown infection with or without petiole lesions; 6 = plant dead, crown necrotic. Plants receiving a DSR of 2.0 or less 30 days after inoculation were considered resistant; those receiving a rating of 4.0 or greater were considered susceptible, while those with a rating between 2.1 and 3.9 were considered intermediate.

Plants inoculated by crown injection were rated for disease severity within 50 days of inoculation by recording the number of dead and the number of surviving plants. Individual plants which were still alive 50 days after inoculation were considered to be resistant. If 75% or more of the plants of the same cultivar or line survived crown injection inoculation with a given isolate, the cultivar or line was considered resistant to that isolate.

Statistical analyses of data. The SAS statistical package (11) was used to conduct analysis of variance tests. If the F-test indicated significant differences due to treatments, separation of treatment means was by Duncan's multiple range test, least significant difference, or Waller-Duncan test.

RESULTS

Conidial characteristics. The 24 Colletotrichum isolates examined were separated based on the predominant conidial shape in each culture and fell into two groups referred to as 'cylindrical' spored (Fig. 2a) and 'fusiform' spored (Fig. 2b) cultures. The conidial morphology of the cylindrical spored cultures seemed to best match the description of C. fragariae and the fusiform spored cultures the description of C. acutatum.

There were 15 cultures classified as C. fragariae having cylindrical conidia (Table 3). Within these cultures, two variations in conidial shape were recognized: 1) those rounded on one end and tapered to a point on the other end; and 2) those with both ends rounded. The conidia with one end pointed were predominant in all 15 cultures, constituting from 80 to 100% of the conidia in each culture with an average of 89% in all cultures. There were nine cultures classified as C. acutatum having fusiform conidia (Table 3). Within these cultures three variations in conidial shape were recognized: 1) those tapered to a point on both ends; 2) those rounded on one end and tapered to a point on the other end; and 3) those rounded on both ends. The predominant shape in all of these cultures was the one pointed on both ends which constituted 64 to 100% of the conidia in each culture with an average of 87% in all cultures.

All the Colletotrichum isolates in this study produced pink to orange conidia in mass. The range of conidial colors overlaped among isolates of the two species, and therefore, were of no value in distinguishing isolates.

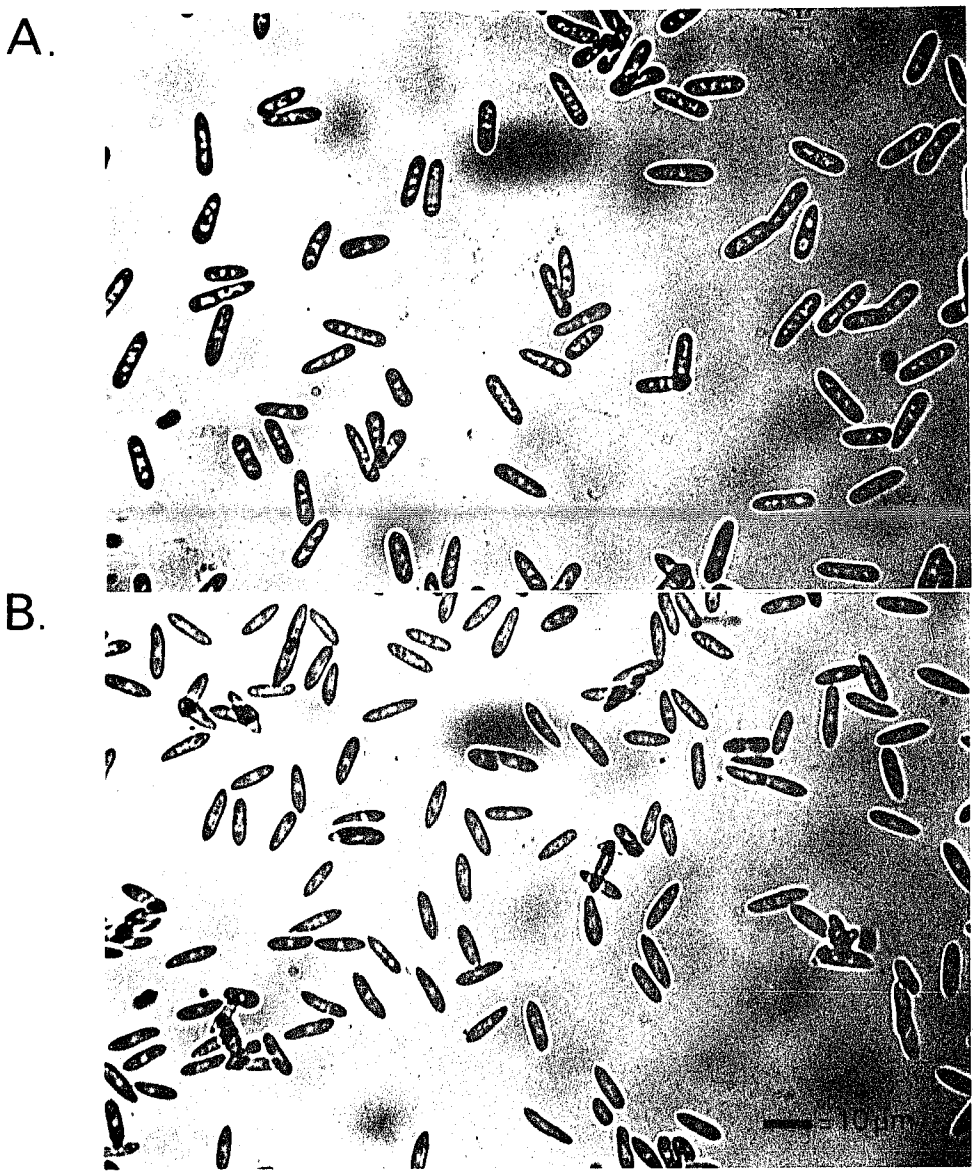


Figure 2. A. Conidia of Colletotrichum fragariae isolate La-2.

B. Conidia of C. acutatum isolate Goff.

Table 3. Morphological and cultural characteristics of Colletotrichum isolates from strawberry plants

Isolate	Conidial Shape	Setae		Color of Colony
		In Culture	On Plant	
		C. fragariae		
Ark P-1	Cylindrical[a]	-[b]	-[c]	Gray[d]
CF-56	Cylindrical	+	+	Olive, dark gray
La-1	Cylindrical	+	+	Olive, dark gray
La-2	Cylindrical	-	+	Olive, dark gray
CF-75	Cylindrical	+	+	Olive, dark gray
CF-63	Cylindrical	-	+	Brown, gray
MS-9	Cylindrical	+	+	Olive, dark gray
CG-163	Cylindrical	-	+	Olive, gray, black
CF-card	Cylindrical	+	+	Olive, gray, white
CG-164	Cylindrical	-	+	Olive, dark gray
CF-4	Cylindrical	+	+	Beige
Fla-1	Cylindrical	+	+	Olive, dark gray
CG-162	Cylindrical	-	-	Olive, gray
Fla-2	Cylindrical	-	+	Olive, gray
CF-1	Cylindrical	-	+	Olive, gray
		C. acutatum		
Cal B	Fusiform[e]	-	-	Orange, dark brown
Cal A	Fusiform	-	-	Orange, brown, white
Mil-2	Fusiform	-	-	Pink, orange, brown
Goff	Fusiform	-	-	Orange, brown
Ark C-1	Fusiform	-	-	Beige, orange, brown
Cal D	Fusiform	-	-	Beige, orange, brown
Cal C	Fusiform	-	-	Beige, pink, gray
Mil-1	Fusiform	-	-	Pink, orange, brown
CF-167	Fusiform	-	-	Rose, olive, gray

[a]Eight-nine percent of the conidia of the 15 isolates were cylindrical with one end tapered to a point and one end rounded, the others were rounded on both ends.

[b]Presence (+) or absence (-) of setae on 5 to 20 day old cultures on PDA or PDA:OMA.

[c]Presence (+) or absence (-) of setae in anthracnose petiole lesions on Albritton, Tangi, or Tioga strawberry cultivars, 10 to 20 days after wound inoculation.

[d]Colony color of 8-day-old petri plate on PDA incubated under alternating near UV light (12 hr) and dark (12 hr).

[e]Eighty-seven percent of the conidia of the nine isolates were fusiform, i.e. tapered to a point on both ends; 10% were rounded on one end and tapered on the other end, and 3% were rounded on both ends.

The total sizes of the conidia, expressed by volume, from 10 of 15 C. fragariae isolates were not significantly different (Table 4). The conidia of isolates Ark P-1 were significantly larger and the conidia of isolates CG-162, Fla-2, and CF-1 significantly smaller than those of the other 12 isolates. When the size of the conidia from the 15 C. fragariae isolates are expressed as a mean for each isolate, the length range is 12.4 to 16.1 μm and the width range is 4.4 to 5.4 μm (Table 4). About half the C. fragariae isolates produced conidia which were shorter on the average than the lower end of the length range reported in the original description by Brooks (2), but the width of the conidia of these isolates fell within the reported range.

The total sizes of the conidia expressed by volume, from seven of the nine C. acutatum isolates were not significantly different (Table 4). Conidia from the nine C. acutatum isolates, expressed as a mean for each isolate, ranged from 12.3 to 13.2 μm long and from 4.6 to 5.3 μm wide (Table 4). The length measurements of the conidia from the C. acutatum isolates are within the range of that reported by Simmonds for C. acutatum; however, all are wider than those in the type description (30).

Appressorial characteristics. Appressorial shape was determined from appressoria formed by germinating conidia because only a few of the isolates produced mycelial appressoria. The C. fragariae isolates usually produced appressoria two to three days sooner than did the C. acutatum isolates. There were about twice as many appressoria per slide culture formed by the C. fragariae isolates as were formed by the C. acutatum isolates. Generally, the C. fragariae isolates' appressoria were slightly more lobed or clavate (Fig. 3a) than the appressoria of

Table 4. Range of means and average size[a] of conidia of 15 isolates of Colletotrichum fragariae and 9 isolates of C. acutatum

Isolate	Volume[b] (μm^3)	Length (μm)		Width (μm)	
		Range[c]	Mean	Range[c]	Mean
			C. fragariae[d]		
Ark P-1	309.9 a[e]	(16-17)	16.1 a	(5-6)	5.4 a
CF-56	262.3 b	(13-14)	13.7 cdef	(5-6)	5.2 abc
La-1	248.4 bc	(14-17)	14.4 bc	(5-6)	5.1 bc
La-2	243.1 bc	(13-16)	15.0 b	(4-5)	4.9 c
CF-75	231.4 bc	(12-15)	13.8 cde	(5-6)	5.0 bc
CF-63	230.9 bc	(13-15)	14.1 bcd	(4-5)	5.0 bc
MS-9	230.0 bc	(13-15)	14.1 bcd	(4-5)	4.9 bc
CG-163	227.0 bc	(13-14)	13.8 cde	(4-5)	5.0 bc
CF-card	226.1 bc	(13-15)	13.2 defg	(5-6)	5.2 ab
CG-164	222.8 c	(13-15)	13.7 cdef	(4-5)	5.0 bc
CF-4	220.9 c	(12-15)	13.2 defg	(4-5)	5.0 bc
Fla-1	220.1 c	(13-14)	13.3 def	(5-6)	5.0 bc
CG-162	171.3 d	(13-14)	12.9 efg	(4-5)	4.4 d
Fla-2	171.2 d	(12-13)	12.9 fg	(4-5)	4.5 d
CF-1	155.5 d	(12-13)	12.4 g	(4-5)	4.4 d
			C. acutatum[f]		
Cal-B	219.9 a[e]	(12-14)	13.2 abc	(5-6)	5.3 a
Cal-A	210.1 ab	(12-14)	13.4 abc	(5-6)	5.2 ab
Mil-2	196.8 ab	(14-16)	14.7 a	(4-5)	4.7 c
Goff	194.8 ab	(13-16)	14.3 ab	(4-5)	4.8 bc
Ark C-1	188.4 ab	(12-15)	13.7 abc	(4-5)	4.8 bc
Cal-D	183.9 ab	(13-14)	13.0 bc	(4-6)	5.0 abc
Cal-C	174.1 ab	(12-15)	13.9 abc	(4-6)	4.6 c
Mil-1	159.3 ab	(12-15)	13.4 abc	(4-5)	4.6 c
CF-167	135.7 b	(9-14)	12.3 c	(4-5)	4.6 c

[a]Conidia were measured in a water mount made from 9 to 13 day old cultures grown on PDA.

[b]Volume (V) calculated from length (ℓ) and width (w) for C. fragariae as $V = 0.25\pi w^2 (\ell - 0.5w)$ and for C. acutatum as $V = 0.25\pi w^2 (\ell - 0.67w)$.

[c]Range of means. Measurements from six sets of conidia; 15 conidia/set.

[d]C. fragariae original description: length range 14 to 21 μm (av. 16.4 μm); width range 3.9 to 6.3 μm (av. 4.8 μm). In reference number 2.

[e]Mean separation within columns within species by LSD (0.05).

[f]C. acutatum original description: length range 8.3 to 14.4 μm (av. 11.1 μm); width range 2.5 to 4.0 μm (av. 3.1 μm). In reference number 29.

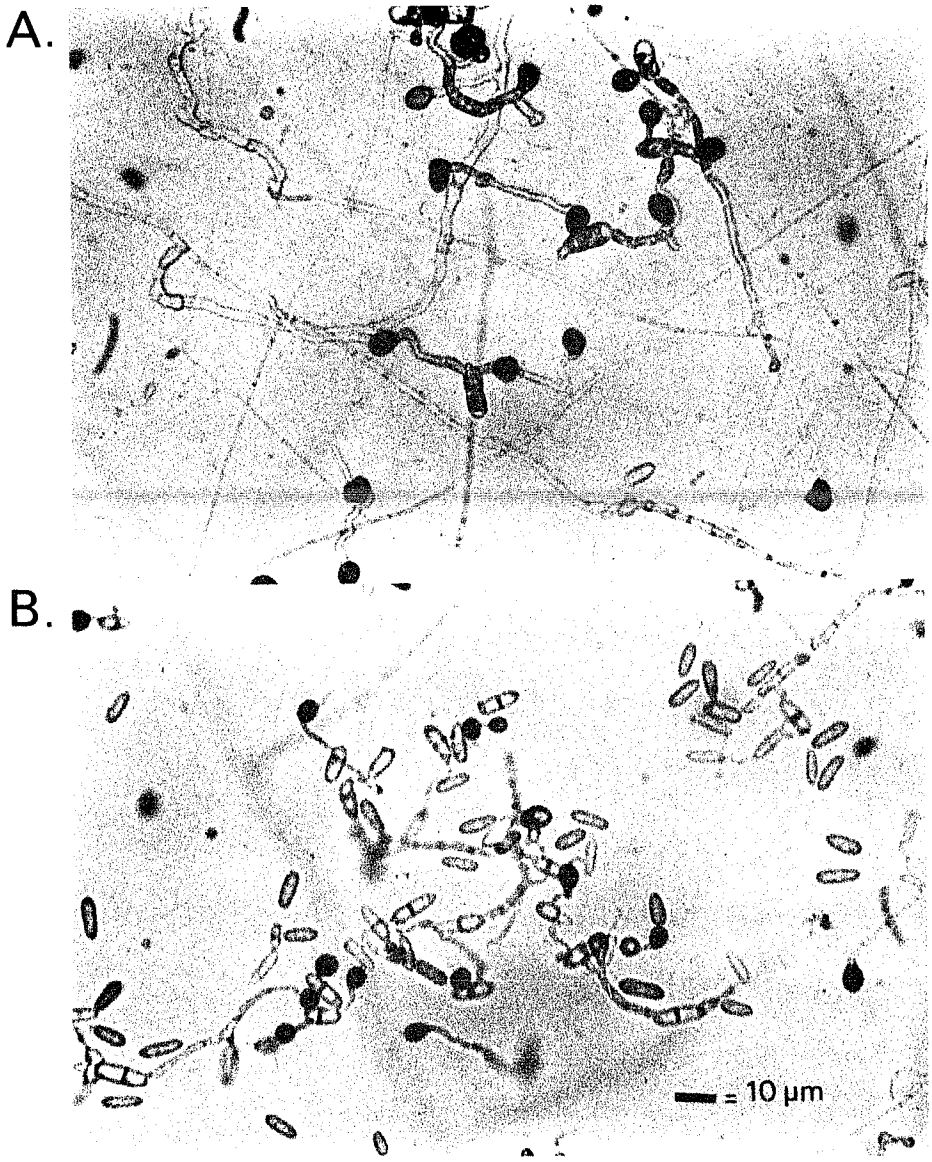


Figure 3. A. Appressoria of Colletotrichum fragariae isolate CF-card.

B. Appressoria of C. acutatum isolate Cal C.

the C. acutatum isolates (Fig. 3b). This difference was not definitive due to considerable overlapping of appressorial types between the two species. Simmonds (30) described the appressoria of C. acutatum as sparse, obovate, rarely lobed. No description of the appressoria of C. fragariae was found.

Setae production. None of the C. acutatum isolates produced setae in culture or on the host (Table 3, Fig. 4b) which agrees with the description of C. acutatum (30, 37). All of the C. fragariae isolates produced setae on the host except for isolate CG-162 which produced the perfect stage on the host as well as in culture and isolate Ark P-1 which produced the Colletotrichum stage on the host and the perfect stage in culture. The setae were 1 to 2 septate and few to abundant on the host depending on the isolate. The mean length of the setae produced on Tioga averaged 72 µm for all 13 C. fragariae isolates which produced setae and ranged from an average length of 45 µm for isolate CF-card to 107 µm for isolate La-1. The mean width of the setae of all 13 isolates was 4.3 µm and ranged from 3.7 µm for isolate CF-4 to 5.6 µm for isolate CF-1.

Setae of several of the C. fragariae isolates functioned as conidiophores under certain conditions (Fig. 5). Within the same acervulus, conidial production occurred both at the tips of setae and on short, hyaline conidiophores (Fig. 4a). Sporulation at the tip of setae was enhanced by incubating excised infected petioles in a moisture chamber for 24 to 48 hr. During this incubation period, setae would become hyaline at the tip and begin producing conidia.

Cultural characteristics. The color of the C. fragariae cultures varied from olive green to dark gray when viewed from above (Fig.6a),

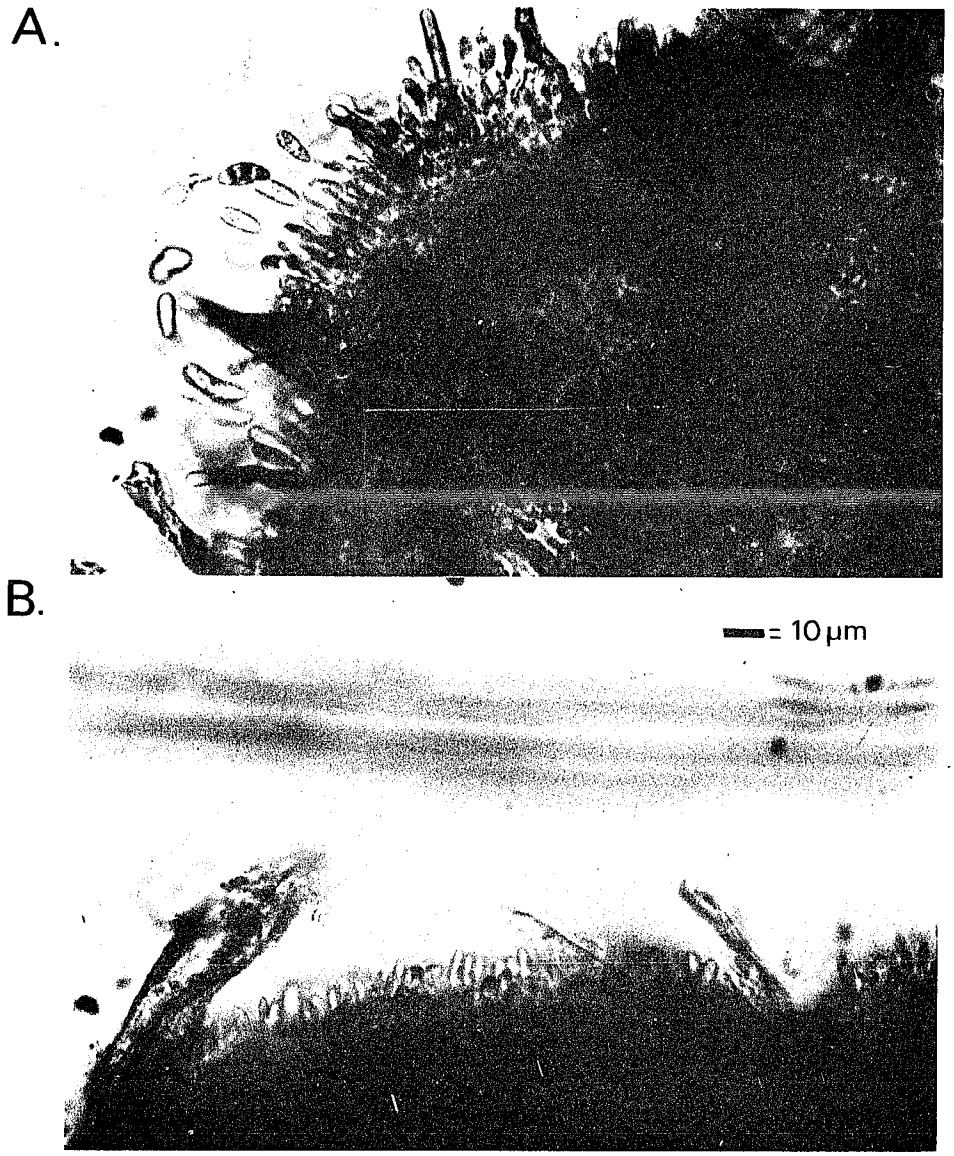


Figure 4. A. Acervulus of Colletotrichum fragariae isolate Fla-1 on Albritton. B. Acervulus of C. acutatum isolate Mil-2 on Tioga.

Figure 5. Sporulating setae of Colletotrichum fragariae isolate Fla-1 on Tioga.

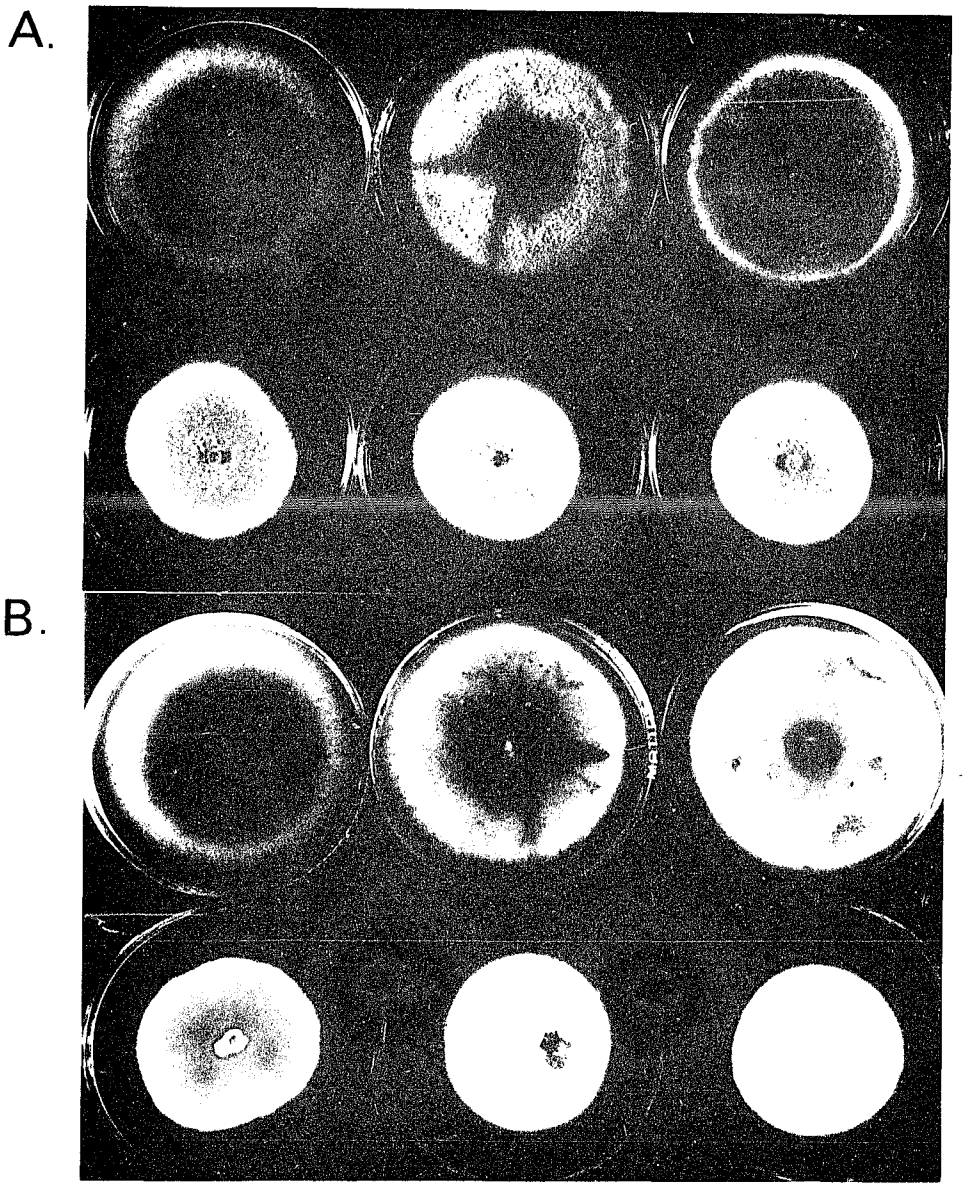


Figure 6. Growth of Colletotrichum fragariae isolates CF-4, CF-63 and CF-75 (top row, left to right) and C. acutatum isolates CF-167, Goff, and Mil-1 (bottom row) on potato dextrose agar five days after 5 mm dia. mycelial plug transfer. A. Cultures viewed from the surface. B. Cultures viewed from the reverse.

while the C. acutatum isolates were usually white during the first few days of growth and later became pink or orange or beige with a pink to orange reverse except isolate CF-167 which was a rose color in culture later becoming gray with a rose reverse (Table 3 and Fig. 6b). The C. fragariae isolates grew faster than the C. acutatum isolates based on a visual index of mycelial development from spores in a plate culture (Table 5 and Fig. 6). The only exception was the Ark P-1 isolate of C. fragariae that grew at the same rate as the C. acutatum isolates.

Tissue susceptibility. All isolates caused an indistinguishable anthracnose fruit rot (Table 6) and reisolation from the fruit lesions always yielded an isolate with characteristics similar to the one used to inoculate the fruit. All of the C. fragariae isolates caused leaf spots following wound inoculation on the two cultivars, Tangi and Tioga, but none of the C. acutatum isolates caused leaf spots (Table 6). All of the C. fragariae isolates except Ark P-1 caused petiole lesions following wound inoculation of the same cultivars while only four of the nine C. acutatum isolates caused petiole lesions (Table 6).

Cultivar-isolate interaction after plant spray inoculation. The C. fragariae isolates caused higher mean disease severity ratings (DSRs) than did the C. acutatum isolates (Table 7). C. fragariae isolate CF-63 caused the highest overall DSR. Of the 15 strawberry lines tested Surecrop received the highest overall DSR in response to both the C. fragariae and C. acutatum isolates while MSUS 70 received the lowest overall DSR to the C. fragariae isolates and Apollo received the lowest DSR to the C. acutatum isolates. Isolate CF-167 did not cause a susceptible reaction (DSR $\geq$ 4.0) on any cultivar tested. No cultivar or line was susceptible to all C. fragariae or C. acutatum isolates, nor

Table 5. Growth[a] of Colletotrichum fragariae and C. acutatum isolates on acidified potato dextrose agar

C. fragariae	Growth Index[b]	C. acutatum	Growth Index
CG-163	9.3[c]	Cal C	6.9
CF-card	9.2	CF-167	6.7
Fla-1	9.0	Cal B	6.5
CG-164	8.9	Cal D	6.1
CF-1	8.9	Cal A	6.0
MS-9	8.8	Mil-1	5.8
La-1	8.7	Ark C-1	5.6
CF-63	8.5	Goff	5.4
CF-75	8.5	Mil-2	5.3
CF-56	8.4		
CG-162	8.3		
Fla-2	8.2		
CF-4	7.6		
La-2	7.3		
Ark P-1	6.6		
Mean	8.4	Mean	6.0

[a]Relative growth of isolates rated on a visual scale of 0 = no growth to 10 = 100% of agar surface covered by mycelium 5 days after agar surface was seeded with a conidial suspension containing 6000 conidia/ml.

[b]Mean growth index of 12 plates of each isolate.

[c]LSD (0.05) = 1.63 for isolate means of both species.

Table 6. Disease reaction of different strawberry tissues to inoculation with a conidial suspension from isolates of *Colletotrichum fragariae* and *C. acutatum*

Isolate	Strawberry Tissue		
	Fruit[a]	Petiole[b]	Leaf[b]
	C. fragariae		
Ark P-1	+	-	+
CF-56	+	+	+
La-1	+	+	+
La-2	+	+	+
CF-75	+	+	+
CF-63	+	+	+
MS-9	+	+	+
CG-163	+	+	+
CF-card	+	+	+
CG-164	+	+	+
CF-4	+	+	+
Fla-1	+	+	+
CG-162	+	+	+
Fla-2	+	+	+
CF-1	+	+	+
	C. acutatum		
Cal B	+	-	-
Cal-A	+	-	-
Mil-2	+	+	-
Goff	+	+	-
Ark C-1	+	-	-
Cal D	+	-	-
Cal C	+	+	-
Mil-1	+	+	-
CF-167	+	-	-

[a]Fruit rot development rated 5 days after inoculation; + = fruit rot and - = no fruit rot.

[b]Disease response of two plants each of Tangi and Tioga recorded 15 days after wound inoculation; + = lesions $\geq$ 3mm and - = lesions < 3mm.

Table 7. Disease severity ratings[a] of 15 strawberry cultivars and lines inoculated with 15 Colletotrichum fragariae isolates and 5 C. acutatum isolates 30 days after plant spray inoculation with a conidial suspension of 1.5 X 10^6 conidia/ml

	Strawberry cultivar or line[b]															
Isolate	SUR	TIO	ALB	TAN	SEQ	FBL	TIT	SUN	CAR	F90	PRE	ROS	TNB	APO	M70	Mean
							C. fragariae isolates									
CF-63	5.0[c]	4.8	5.3	4.8	4.5	3.0	4.3	4.5	3.5	3.8	3.0	4.0	3.8	3.8	1.0	3.92
La-1	5.3	5.8	5.3	4.0	4.0	3.8	4.0	5.0	2.8	3.3	2.5	4.0	1.5	2.3	4.0	3.82
Fla-1	5.5	4.0	5.0	5.5	5.5	3.8	3.5	3.5	4.0	2.5	2.5	5.3	1.8	2.3	2.5	3.80
CF-card	5.3	4.5	4.8	5.0	2.5	4.5	4.5	4.3	3.3	2.0	2.5	3.8	4.5	2.5	1.5	3.68
CF-4	5.3	4.8	4.8	3.8	3.8	3.8	4.5	3.5	3.0	3.8	3.3	3.3	2.3	3.3	1.5	3.62
La-2	5.5	5.3	4.3	3.5	3.5	4.5	3.8	4.3	3.8	2.3	4.3	3.3	1.5	1.3	2.0	3.52
CF-75	4.3	4.0	4.5	3.8	4.0	4.3	5.0	3.8	2.3	2.5	4.0	2.5	2.0	2.3	1.5	3.37
MS-9	4.3	4.5	4.3	3.8	4.3	4.0	3.3	2.8	4.5	2.0	3.5	2.3	1.8	2.0	2.8	3.32
CF-56	5.5	4.5	4.5	4.0	3.3	3.8	3.0	4.0	2.5	3.5	4.0	1.0	3.3	1.3	1.3	3.31
CF-1	4.0	4.5	4.0	4.0	3.8	3.3	2.8	3.3	2.5	4.3	3.3	2.0	2.8	2.0	1.5	3.18
CG-164	4.5	5.8	2.0	3.5	3.5	3.3	2.0	3.8	2.0	3.5	2.3	0.5	5.5	2.5	2.0	3.10
Fla-2	6.0	4.0	4.3	4.5	3.5	2.8	4.3	1.5	2.0	2.3	1.5	3.0	2.0	2.0	2.3	3.05
CG-163	4.3	4.5	1.8	4.5	4.3	2.5	2.8	3.5	4.5	3.5	3.0	0.5	1.5	2.5	2.0	3.03
CG-162	4.8	4.0	5.0	2.3	4.3	3.3	4.0	2.3	2.0	2.3	2.3	1.3	2.5	1.3	1.0	2.82
Ark P-1	2.5	2.0	3.3	1.3	1.0	4.0	1.3	2.0	1.5	2.5	1.5	3.0	2.8	0.5	0.8	1.98
	LSD (P = 0.05) for host-pathogen combination = 1.795															
Mean	4.78	4.47	4.18	3.87	3.70	3.62	3.52	3.45	2.93	2.92	2.88	2.63	2.62	2.10	1.83	3.30
							C. acutatum isolates									
Goff	5.0	4.0	4.0	3.3	3.0	3.8	3.5	4.8	2.5	3.5	3.0	2.8	3.8	2.8	2.8	3.48
Mil-1	5.0	3.5	3.5	5.0	3.5	3.5	2.8	5.0	2.3	1.5	2.8	2.3	4.0	1.3	2.8	3.23
Mil-2	4.8	4.0	4.0	2.0	3.5	3.0	4.5	3.8	3.5	1.8	2.8	2.8	2.5	1.5	2.3	3.10
Ark C-1	4.8	1.8	3.8	1.0	1.8	4.0	3.3	3.8	3.0	0.3	2.3	3.5	3.3	0.3	0.5	2.47
CF-167	3.8	2.3	2.0	1.8	3.0	0.0	1.0	1.8	0.5	0.8	0.8	0.0	0.8	1.0	1.3	1.37
	LSD (P = 0.05) for host-pathogen combination = 1.876															
Mean	4.65	3.10	3.45	2.60	2.95	2.85	3.00	3.80	2.35	1.55	2.30	2.25	2.85	1.35	1.90	2.73

Footnotes on next page.

Table 7. Continued

[a]Disease severity rating (DSR) scale: 0 = no symptoms to 6 = plant dead.

[b]SUR, Surecrop; TIO, Tioga; ALB, Albritton; TAN, Tangi; SEQ, Sequoia; FBL, Florida Belle; TIT, Titan; SUN, Sunrise; CAR, Cardinal; F90, Florida Ninety, PRE, Prelude; ROS, Rosanne; TNB, Tennessee Beauty; APO, Apollo; M70, MSUS 70.

[c]Average DSR of four plants.

was any resistant (DSR $\leq$ 2.0) to all isolates. C. fragariae, isolate La-1, caused a susceptible response on more of the cultivars (9 of 15) than any other isolate while isolate Ark P-1 caused a susceptible response on only one cultivar. Some cultivar-isolate pairs resulted in opposite responses, i.e. isolates CG-163 and Fla-2 on Albritton and Cardinal.

Ten pathogenic races were identified among 13 C. fragariae isolates (Table 8) based on the disease response of six strawberry cultivars and a breeding line (Table 7). Surecrop was included among the differential hosts because it was susceptible to all races. The other five cultivars and the breeding line were chosen because of their resistant (DSR $\leq$ 2.0) response to the various races. Tennessee Beauty was the only differential host resistant to isolates of Race 9 while line MSUS 70 was the only differential host resistant to isolates of Race 10. The other races were identified based on a combination of differential hosts resistant to each race. Isolates CG-162 and Ark P-1, both of which produce the G. cingulata ascigerous stage in culture, were not included in the race separation. Several of the cultivars which were resistant to certain races were not included in the differential hosts because their disease response did not add to the race determinations.

Cultivar-isolate interaction after crown injection inoculation. The six C. fragariae isolates caused more severe reactions when injected into the crown of plants of strawberry cultivars and lines than did the two C. acutatum isolates tested (Table 9). However, both C. acutatum isolates did cause crown rot on some cultivars and lines. Rosanne and MSUS 27 were susceptible to crown infection by C. acutatum

Table 8. Identification of *Colletotrichum fragariae* races by disease reactions[a] of differential hosts following plant spray inoculation

Race	Isolate	Strawberry Cultivar or Line[b]						
		SUR	SUN	F90	ROS	APO	TNB	M70
1	Fla-2	+	R	+	+	R	R	+
2	CF-75	+	+	R	+	+	R	R
3	CF-1	+	+	+	R	R	+	R
	CF-56	+	+	+	R	R	+	R
4	CG-163	+	+	+	R	+	R	R
5	La-2	+	+	+	+	R	R	R
6	CF-card	+	+	R	+	+	+	R
7	CG-164	+	+	+	R	+	+	R
8	MS-9	+	+	+	+	R	R	+
9	Fla-1	+	+	+	+	+	R	+
	La-1	+	+	+	+	+	R	+
10	CF-4	+	+	+	+	+	+	R
	CF-63	+	+	+	+	+	+	R

[a]Based on disease severity ratings (DSRs) in Table 7: R = resistant, DSR $\leq$ 2.0; + = intermediate or susceptible, DSRs >2.0.

[b]SUR = Surecrop, SUN = Sunrise, F90 = Florida Ninety, ROS = Rosanne, APO = Apollo, TNB = Tennessee Beauty, M70 = MSUS 70.

Table 9. Disease response[a] of four strawberry cultivars and three lines 50 days after crown injection with isolates of Colletotrichum fragariae and C. acutatum

Isolate	Strawberry cultivar or line						
	Ros-anne	Tan-gi	Tenn. Beauty	MSUS 27	Car-dinal	MSUS 70	MSUS 42
			C. fragariae isolates				
CG-164	+	+	+	+	-	+	+
CG-163	+	+	+	+	+	+	-
CF-63	+	+	+	+	+	+	-
CF-card	+	+	+	+	+	-	-
Fla-2	+	+	+	+	+	-	-
CF-1	+	+	+	-	+	-	-
			C. acutatum isolates				
Mil-2	-	+	-	-	+	+	-
CF-167	+	-	-	+	-	-	-

[a]Based on response of four plants. Resistant (-) = three or four plants survived; susceptible (+) = none to two plants survived.

isolate CF-167, which was obtained from a rotten fruit in a field in Florida from plants that had been grown in an Arkansas nursery. Tangi, Cardinal, and MSUS 70 were susceptible to C. acutatum isolate Mil-2 which was taken from a Cardinal fruit lesion in a Mississippi fruit production field in which plants were also dying of crown rot from which a similar isolate, Mil-1, was obtained. All C. fragariae isolates caused susceptible reactions on all commercial cultivars that were crown injected except isolate CG-164 on Cardinal; however, line MSUS 70 was resistant to three of the C. fragariae isolates and line MSUS 42 to all C. fragariae isolates except CG-164.

When the disease response of the five strawberry lines to the six C. fragariae isolates and the two C. acutatum isolates which were included in both the plant spray study (Table 7) and the crown injection study (Table 9) were compared, the response of most plants to crown injection was as severe or more severe than their response to plant spray inoculation. Any line rated susceptible to an isolate based on its petiole reaction to plant spray inoculation was also rated susceptible to that isolate based on its reaction to crown injection. More importantly, over half of the strawberry line-isolate combinations were rated susceptible to crown injection (Table 9) after having been rated resistant (DSR $\leq$ 2.0) in the plant spray inoculation test (Table 7). Furthermore, most of the strawberry line-isolate combinations were rated susceptible to crown injection after having been rated intermediate (DSR 2.1 to 3.9) in the plant spray inoculation test.

DISCUSSION

Based on conidial morphology, presence or absence of setae, colony color, and growth rate in culture, the 24 Colletotrichum isolates studied were placed into two Colletotrichum species, C. fragariae and C. acutatum. Brooks (2) described C. fragariae conidia as spindle to boat-shaped, but in his photomicrograph and drawing the conidia appear to be of two types; 1) cylindrical with one end rounded and the other tapered to a point and 2) cylindrical with both ends rounded. The C. fragariae isolates in this study were predominately of the first shape (Fig. 1a). Conidial shape is a diagnostic feature of C. acutatum and is described as fusiform (37). All the isolates with fusiform conidia in this study were placed in C. acutatum even though all produced conidia wider than those in the type description. Simmonds (30) included a photomicrograph of a "larger spored form" of C. acutatum in his report containing the original description of the species, but did not report its size. Isolates Ark P-1 and CG-162, which are listed with the C. fragariae isolates, produced the perfect stage (G. cingulata) in culture and CG-162 also produced the perfect stage on the host in greenhouse tests.

In his original description of Colletotrichum fragariae, Brooks (2) described the setae as sometimes having a constricted, apical cell. His drawing appears somewhat like some stages of the sporulating setae observed on several of the isolates in this collection. Though no reference was found to sporulating setae in descriptions of C. fragariae, this is a typical feature of most of the C. fragariae isolates in our collection. A photomicrograph by Milholland (27) and a

drawing by Mora et al (26) both show what appear to be sporulating setae. In his description of Glomerella cingulata, Mordue (28) indicates that conidia are occasionly borne on the setae.

The ten pathogenic races of C. fragariae that were present in our small collection indicates that many races may be present in the southeastern United States. Both vertical and horizontal resistance to C. fragariae appear to be present in the strawberry lines in this study as indicated when isolates are ranked by their DSRs within each host line as suggested by Vanderplank (38) as a test for vertical resistance. The order of isolates within a line is not the same on each host line indicating the presence of vertical resistance, but generally the isolates listed first on Table 7 cause a more severe reaction on most lines than do the isolates listed last indicating the presence of horizontal resistance. If the host lines are ranked within each isolate, the same response is apparent.

The results of the crown injection study show that while most cultivars were extremely susceptible to crown infection some strawberry lines posses resistance to crown infection by C. fragariae, but this resistance does not always correlate with resistance to petiole infection. Rosanne, which is resistant to petiole infection by most isolates, was very susceptible to crown infection by many of these same isolates. Line MSUS 70 was resistant to both crown and petiole infections.

C. acutatum had previously been reported to cause fruit rots (30, 35, 36) and stolon and petiole lesions (7) of strawberries but not crown rot. Two of the C. acutatum isolates in this collection were from the crown of plants wilting in the field and two of the fruit isolates

caused crown infection when injected into the crown of several cultivars, thus showing the involvement of C. acutatum in crown rot as well as fruit rots. Interestingly, all the C. acutatum isolates in this study except for the four California isolates were cultured from plants that had been grown in Arkansas nurseries.

The pathogenic variation among isolates of C. fragariae must be considered when choosing isolates to use in an anthracnose-crown rot screening program. Isolates such as La-1, CF-63, Fla-1, or CF-card which caused a susceptible reaction on eight or nine of the 15 cultivars and lines would be reasonable choices from among the isolates in this collection. Most cultivars responsed similarly to the C. fragariae and the C. acutatum isolates with cultivars more susceptible to C. fragariae also being more susceptible to C. acutatum and those more resistant to C. fragariae also being more resistant to C. acutatum.

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VITA

Barbara Jones was born March 3, 1950 in Phenix City, Alabama and moved with her parents to a small farm in Stone County, Mississippi later that year. She attended public schools in Stone County and Mississippi Gulf Coast Junior College at Perkinston, Mississippi before transferring to Mississippi State University where she worked in the Department of Plant Pathology and Weed Science while studying for her degrees. She received her Bachelor of Science Degree in Plant Pathology and Weed Science in January, 1972 and her Master of Science Degree in Plant Pathology and Weed Science in May 1973 from Mississippi State University. In 1971 she married Robert D. Smith, and they are the parents of two boys, Robert Decker and Joseph Scott. From 1973 until 1975 Mrs. Smith worked as laboratory technician at the South Mississippi Branch Experiment Station in Poplarville, MS, and during 1976 taught biology at Southeastern Baptist College in Laurel, MS. In 1977 she was employed as Plant Pathologist at the USDA-ARS Small Fruit Research Station in Poplarville, MS. Her major research responsibility was to develop a disease screening program to identify antracnose-crown rot resistant strawberry seedlings from a regional breeding program. While employed at the Small Fruit Research Station, Mrs. Smith attended Louisiana State University from 1980 until she completed her degree in 1985. She remains employed at the Small Fruit Research Station conducting research on diseases of blueberry, blackberry and strawberry.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Plant Pathology

Title of Dissertation: Strawberry Response to Colletotrichum fragariae and Colletotrichum acutatum

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